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FORECASTING LATE BLIGHT EPIPHYTOTICS OF POTATOES AND TOMATOES[1]

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INTRODUCTION

A method of forecasting epiphytotics is essential to the practical and profitable use of sprays and dusts for plant-disease control. The routine application of fungicides that is so often recommended is economically sound only for those areas where disease losses occur with sufficient regularity so that the benefits over a period of years will profitably counterbalance the cost of spraying or dusting every year.

Even such a disease as late blight of potatoes is usually epiphytotic in less than 50 percent of the years. Lutman (*9*)[3] reports it important in only 7 out of 20 years in Vermont, Martin (*10*) in only 13 out of 34 in New Jersey, Moore (*12*) in only 5 out of 20 in South Carolina, and in eastern Virginia it has caused serious damage in only 2 years in the last 31. It is obvious that tremendous savings in time and money and better disease control would be obtained if the growers were able to dispense with spraying or dusting in years when blight is not likely to cause serious damage and yet treat extra thoroughly in years when the disease threatens to develop. Late-blight forecasting in areas where anthracnose is serious would make it possible to use the fungicides that prevent anthracnose until late blight threatened to develop instead of the alternating spray schedule.

A method of forecasting late blight in eastern Virginia was developed following the epiphytotic in 1946 (*5*) and was given a practical test in 1947 (*6*). The forecasts in 1947 indicated correctly that late blight would not be important and enabled the growers to dispense with needless spraying or dusting. The potential savings from not having to spray or dust the 30,000 acres each of potatoes and tomatoes are estimated to have amounted to approximately $2,000,000 in 1947.[4]

The forecasting method developed for eastern Virginia is new and is not perfect. It was modified to some extent during the 1947 season, and further improvements are anticipated. In its present form, it is about 84 percent accurate for eastern Virginia, and preliminary studies indicate that the basic principles of this method may be used in other areas.

[1] Received for publication January 24, 1947.

[2] The author wishes to express his appreciation to T. J. Nugent, associate plant pathologist, for suggestions and criticism throughout the progress of this work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 563.

[4] Based on six to seven applications at $5 an application.

REVIEW OF LITERATURE

The literature on the relation of late blight to the climatic conditions is extensive, and attempts, with varying degrees of success, have been made to forecast epiphytotics. Lutman (*9*) chartered the weather in relation to late-blight epiphytotics in Vermont for the 20-year period from 1891 to 1910 and concluded that the chief factor was rainfall, but that it is limited in its effects by temperature, sunshine, and probably wind. Martin (*10*) concluded from a study of the weather for the 34 year period from 1889 to 1922 that late blight epiphytotics in New Jersey were favored by wet, cool weather in July. Samson (*13*) chartered the 60-year Indiana State average temperature and rainfall for June, July, August, and September and compared them with the temperature and rainfall for the northern and southern parts of the State in 1945 and 1946 when late blight occurred. He concluded that serious blight may develop when mean temperatures range below normal and rainfall is slightly to considerably above normal in one or more of the months of July, August, and September.

Van Everdingen (*14*) defined four climatic conditions that are essential to the development of a late blight epiphytotic:

(1) Night temperature below the dewpoint for at least 4 hours;
(2) Minimum temperature of 10°C. (50°F.) or above;
(3) Mean cloudiness on the following day of 0.8 or above;
(4) Rainfall during the next 24 hours of at least 0.1 mm.

These four conditions were confirmed by Van Poeteren (*15*) and made the basis for a late-blight warning service in Holland in 1928. Wiltshire (*16*) studied the Dutch data and found that the four conditions laid down by Van Everdingen were in close agreement with the climatic conditions preceding blight epiphytotics in England. Beaumont, Hodson, and Stanilund (*1*) also confirmed Van Everdingen's findings and in addition noted that a high relative humidity (75 percent or above) was an important influencing factor. Beaumont and Stanilund (*2*) noted that a high relative humidity at 3 p. m. indicated a high relative humidity all day and concluded that an epiphytotic was indicated if the relative humidity was not below 75 percent at 3 p. m. for two successive days and the temperature was not too low. They (*3*) set up the following five conditions necessary to count a day favorable for late blight:

(1) Dew either the night before or in the morning;
(2) Minimum temperature of 50° F. or above;
(3) Sunshine less than 5 hours;
(4) Rainfall at least 0.01 inch;
(5) Relative humidity at 3 p. m. not less than 75 percent.

Later they (*4*) reduced the factors for forecasting to two:

(1) Minimum temperature 50° F. or over;
(2) Relative humidity not under 75 percent for at least 2 days.

Moore (*12*) set up a forecasting system for the Charleston, S. C., area based on the total monthly rainfall and number of days with 0.2 inch or more of rain in March, April, and May. He reported that he did not find any correlation with the mean monthly temperature or the relative humidity.

Melhus (*11*) reported on attempts at forecasting late blight for the upper Mississippi Valley States for the years 1941 through 1944.

THE VIRGINIA FORECASTING METHOD

The forecasting method for eastern Virginia is based on a comparison of the average rainfall and average mean temperature of the blight and nonblight years during the 17-year period from 1930 through 1946. This period was used because the writer knew from personal observation when blight occurred and how serious it was in most of these years. The critical period when blight epiphytotics start was determined, and charts were developed by which it is possible to compare the current season's temperature and rainfall with the conditions that favor a late-blight epiphytotic.

The critical temperature above which blight would not be important was considered to be 75° F. This was based on Crosier's findings (*7*) that infection dropped from 89.81 percent at 69.8° (21° C.) to 18.19 percent at 75.2° (24° C.) and to only 2.34 percent at 77° (25° C.). Although sporulation and infection may take place at temperatures over 75°, the amount of infection at the higher temperatures is so small that the fungus would multiply too slowly to cause an epiphytotic. The temperature was expressed as the mean of the 24-hour maximum and minimum. Although such a mean is not so true an expression of the average temperature as an hourly average or thermograph average, as has been shown by Hartzell (*8*), it is the most practical since that is the type of weather data that are usually available for a long period of years.

The critical period when late blight epiphytotics develop was determined by (1) the records of the first appearance of the disease in the blight years, and (2) by means of a graph on which the moving 7-day total rainfall and 7-day average mean temperature were plotted for the blight and nonblight years (fig. 1). On this chart, each point on the rainfall lines represents the total rainfall for the 7-day period ending that day, and each point on the temperature lines represents the average of the mean temperatures of the days during the 7-day period. This chart shows that the temperature was below 75° F. and therefore favorable for the disease until about the middle of June in both blight and nonblight years. It also showed that the rainfall during the last three weeks of May was much heavier in the blight years than in the nonblight years. Blight was observed on June 6 in 1938 and on May 23 in 1946. It was probably present earlier in 1938 but was overlooked until June 6. The differences in rainfall in May for the blight and nonblight years and the dates when blight occurred indicate that the critical period when late-blight epiphytotics start in eastern Virginia is in May. The rainfall lines on the chart diverge on May 14; and since the rainfall plotted on that date represents the total rainfall of the preceding 7 days, the conditions favoring blight are considered to have started about May 8.

A chart (fig. 2) was prepared showing the average cumulative rainfall and average temperature for the critical period (beginning May 8) for the 2 blight years and the 15 nonblight years during the period 1930–46. Then a straight median line was constructed be-

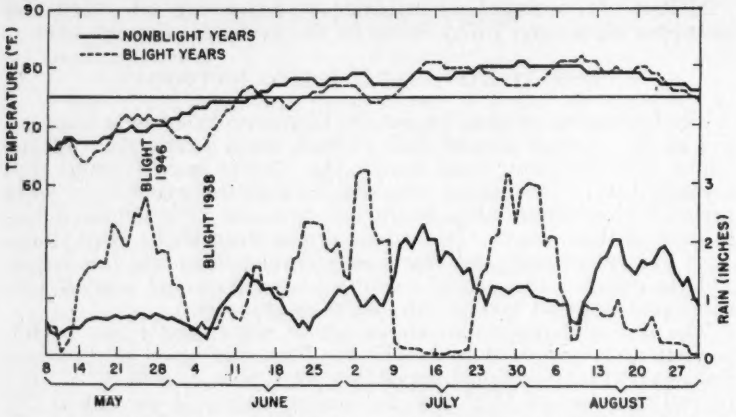

FIGURE 1.—Comparison of the rainfall and temperature of 15 non-blight years and 2 blight years at Norfolk, Va., by means of 7-day moving charts. Each point on the rainfall lines represents the total rainfall for the 7-day period ending that day, and each point on the temperature lines represents the average of the mean temperatures of the days during the 7-day period.

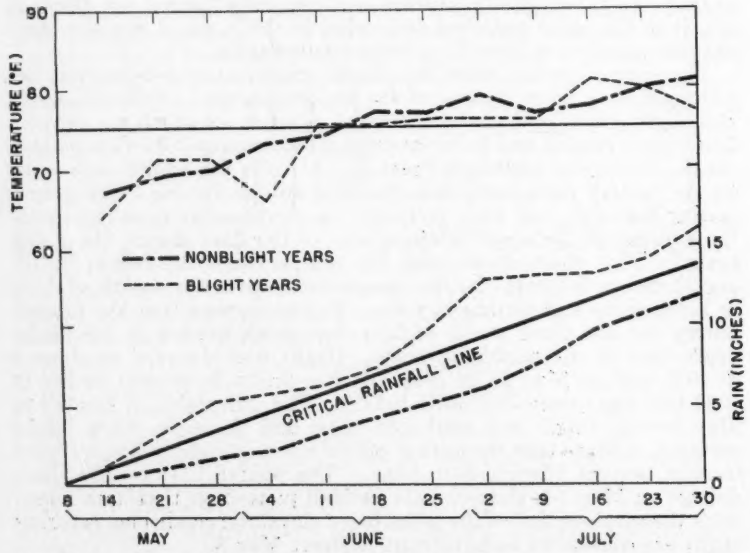

FIGURE 2.—Comparison of the cumulative rainfall and average mean temperatures of 15 nonblight and 2 blight years at Norfolk, Va., in relation to the critical temperature and rainfall lines. These data were plotted at 7-day intervals.

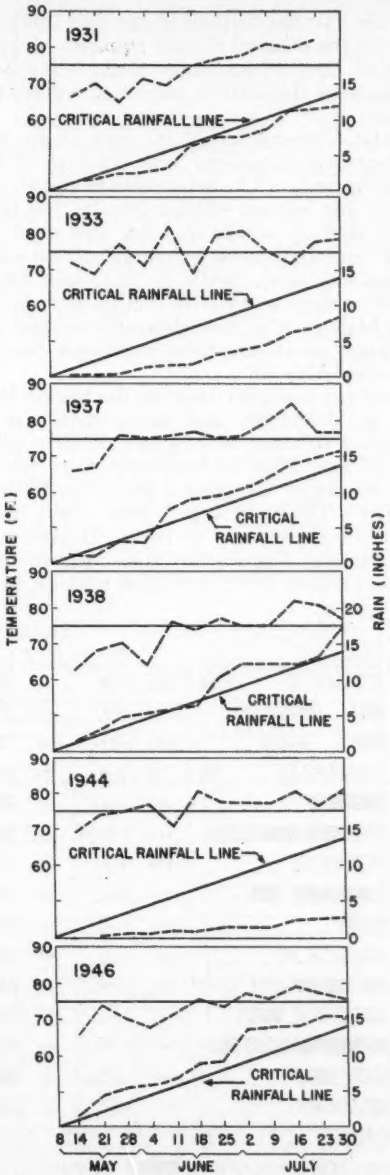

FIGURE 3.—Comparison of the cumulative rainfall and the 7-day moving average temperature of two late-blight years (1938 and 1946) and four nonblight years (1931, 1933, 1937, and 1944) at Norfolk, Va., with the critical rainfall and temperature lines.

tween the lines for rainfall for the blight and nonblight years either by inspection or by the method of least squares. This line, designated the critical rainfall line, is used as a measure for determining if the rainfall at any time in the critical period is sufficient to favor a late-blight epiphytotic.

The rainfall and temperature of the two blight years and four of the nonblight years are compared with the critical rainfall and temperature lines in figure 3. The temperature stayed below 75° F. and the rainfall above the critical rainfall line for the first four weeks in 1938 and for the first six weeks in 1946, the years when blight was serious. In 1931 and 1937 both temperature and rainfall were favorable for only the first week, while in 1933 and 1944 there were no periods of a week or more when both factors were favorable for blight. Although the rainfall in 1937 was above the critical line after June 7, there was no danger of an epiphytotic because the temperature had been above 75° since May 28.

The rainfall and temperature data for the period 1917 through 1947 were analyzed, as described, and the results are summarized in figure 4. According to observations and records, blight was epiphytotic only in 1938 and 1946. Conditions were favorable for blight for the first four weeks of the critical period in 1938 and for the first six weeks in 1946. The only other year when the weather corresponded to those conditions was in 1929. However, the disease was not reported in eastern Virginia for that year in the Plant Disease Reporter or in the station files. It is also difficult to explain the lack

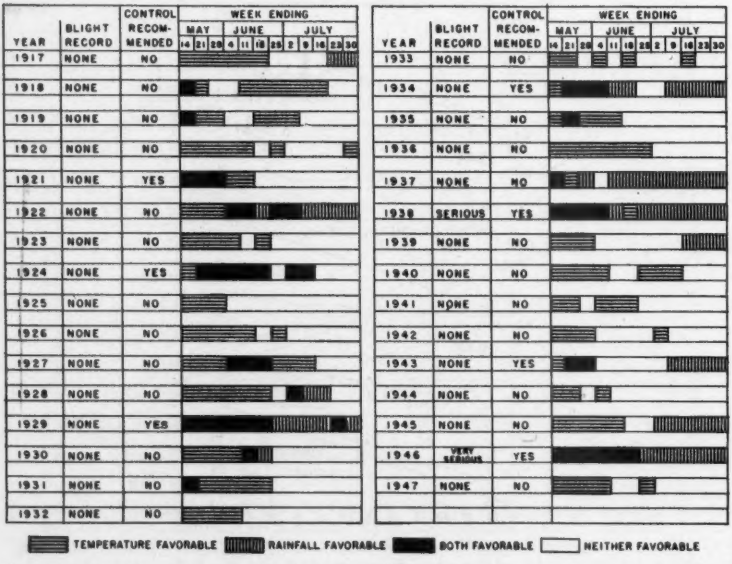

FIGURE 4.—Summary of the temperature and rainfall in relation to the importance of late blight at Norfolk, Va., for the period 1917–47.

of reports on late blight in 1924 except that the rainfall was not favorable until a week later than in 1938 and 1946.

In making forecasts from the chart in figure 4, spraying or dusting would be recommended after two consecutive weeks of favorable temperature and rainfall in May, and recommendations to cease the treatments would be made after 2 weeks of weather unfavorable for blight. On this basis, if the forecasting had been in use since 1917, spraying or dusting would have been recommended in 1921, 1924, 1929, 1934, 1938, 1943, and 1946, or in 7 out of 31 years. Late blight did not cause damage in any of the 24 years when treatment would not have been recommended, but it was serious in 2 and not serious in 5 of the other years. Spraying would have been eliminated in 77 percent of the years, and the predictions would have been 84 percent correct. The only error would have been in spraying in five seasons, when late blight actually threatened but was checked by the intervention of hot or dry weather or other factors before serious damage was done.

USE OF THE FORECASTING METHOD IN OTHER AREAS

In order to see if the forecasting system would work in other areas, the weather data at Charleston, S. C., for April and May for the years 1917 through 1947 were analyzed, and a critical rainfall line was developed for that area. The Charleston area was chosen for this study because it had already been the subject of a forecasting attempt by Moore (*12*), and there were published data on the occurrence of late blight there.

The moving 7-day chart (fig. 5) indicates that the critical period

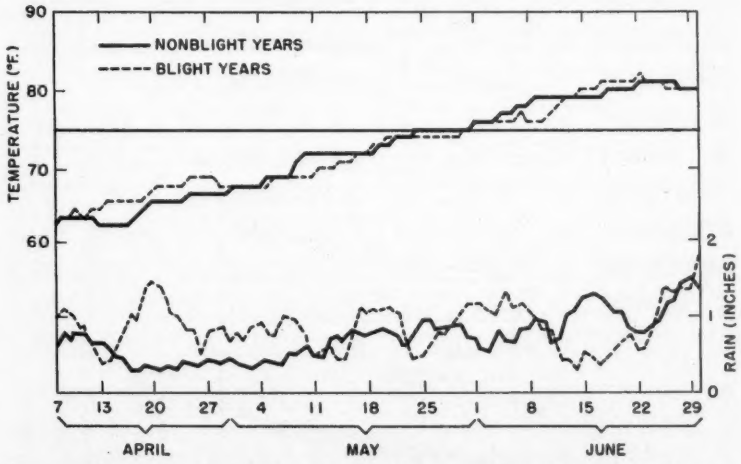

FIGURE 5.—Comparison of the rainfall and temperature of 23 nonblight years and 8 blight years at Charleston, S. C., by means of 7-day moving charts. Each point on the rainfall lines represents the total rainfall for the 7-day period ending that day, and each point on the temperature lines represents the average of the mean temperatures of the days during the 7-day period.

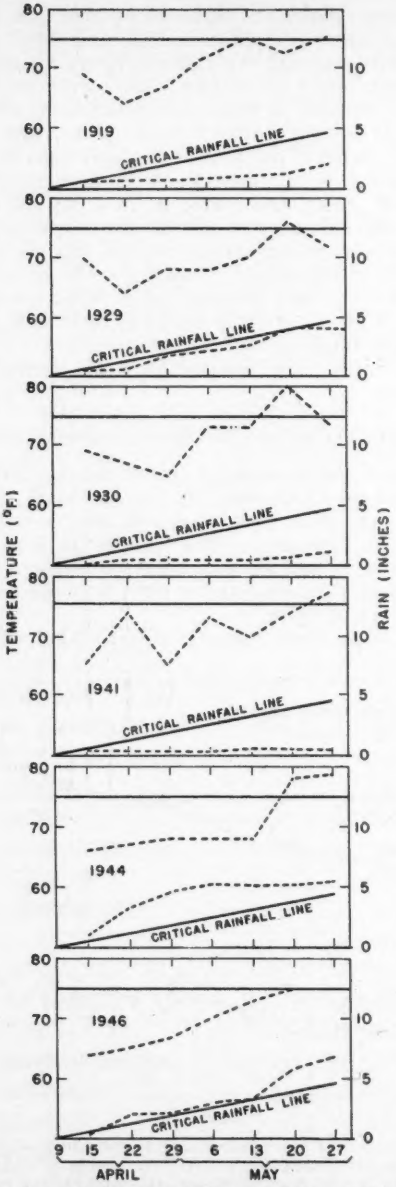

FIGURE 6.—Comparison of the cumulative rainfall and 7-day moving average mean temperature of six typical years at Charleston, S. C., with the critical rainfall and temperature lines for that area. The rainfall was too low for a blight epiphytotic in 1919, 1930, and 1941, was favorable in 1944 and 1946, and intermediate in 1929.

begins about April 9. The rainfall and temperatures for six of the typical years are compared with the critical rainfall and temperature in figure 6.

It is obvious from these charts that the rainfall in 1919, 1930, and 1941 was too low to favor an epiphytotic of late blight, and the rainfall in the blight years 1944 and 1946 was high enough to favor the disease. The year 1929 was a border-line one that would have been difficult to forecast. Blight actually caused serious damage that year even though the rainfall never rose above the critical rainfall line. However, the critical rainfall and 1929 rainfall lines on the chart were nearly parallel and so close together that recommendations to spray or dust would have been justified.

The data for the 31 years are summarized in figure 7. If forecasting for the Charleston area were based on two consective weeks of

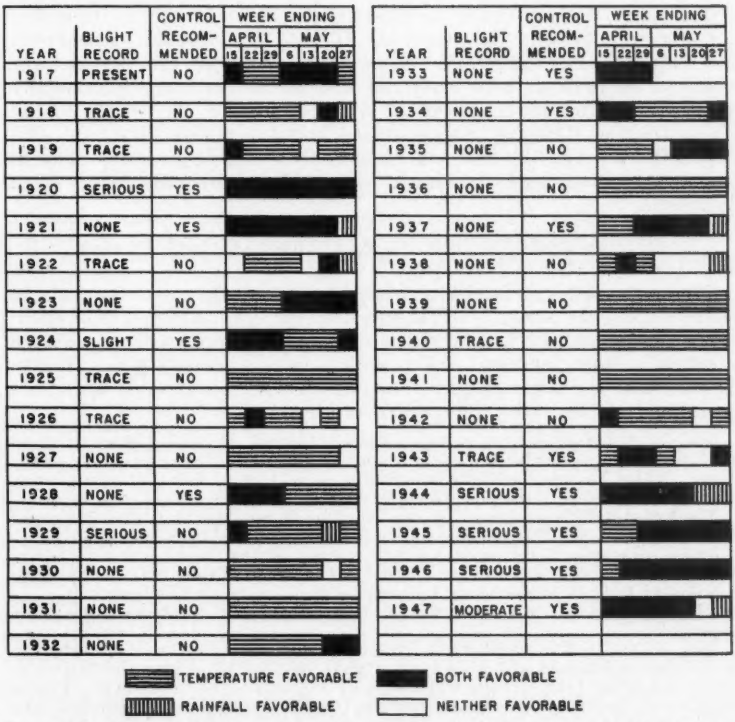

FIGURE 7.—Summary of the temperature and rainfall in relation to the importance of late blight at Charleston, S. C., for the period 1917–1947.

blight-favorable rainfall and temperatures during the period April 8 to May 7, the seasons 1920, 1921, 1924, 1928, 1933, 1934, 1937, 1943, 1944, 1945, 1946, and 1947 would have been considered favorable for the disease, and control measures would have been recommended.

Actually, late blight was epiphytotic or started to be serious in 6 of these 12 years. No blight or only traces of blight were reported in 18 of the other years. The disease caused serious damage in 1929, even though this chart indicates that conditions were unfavorable. However, error in making recommendations for 1929 would have been avoided, since the graph in figure 6 would have shown that it was a border-line year, and control measures would have been recommended.

Forecasts based on the graphs in figure 6 and the chart in figure 7 would have made it possible to eliminate spraying or dusting safely in 18 of the 31 years, or 58 percent of the time. Blight would actually have caused serious damage in 7 of the 13 years when control measures were recommended. The predictions would have been correct in 25 of the 31 years, or 81 percent of the time.

Moore (*12*) designated 1917, 1920, 1922, 1924, and 1929 as serious blight years, but according to the chart in figure 7, the weather was not favorable for an epiphytotic in 1917, 1922, and 1929. Reports in the Plant Disease Reporter [5] show that, although the disease was reported in 1917, it was scattered and never became economically important. This is in agreement with the chart. According to the Plant Disease Reporter Supplement,[6] blight was not found in 1922 until May 23, and the loss was estimated at only 2 percent. This is also in agreement with the chart. The 1929 season has already been discussed.

ACTUAL FORECASTING IN EASTERN VIRGINIA IN 1947

The real test of the forecasting method is to make predictions about blight during the season. This was done in 1947, when it was accurately predicted that late blight would not be epiphytotic in eastern Virginia. Four forecasts were issued to the newspapers, county agents, and the Late Blight Warning Service [7] at weekly intervals beginning on May 15. They were as follows:

May 15, 1947: Weather conditions during the week beginning May 8th have not been favorable for the late blight disease to develop on the potato and tomato crops. There has been only 21 hundredths of an inch of rain during the week which is considerably less than would be necessary for the disease to develop.

The five day forecast issued by the Weather Bureau on May 13th indicated that only about ½ inch of rain is expected between that date and May 18th.

Unless unusually heavy rains occur, it is unlikely that late blight will be important in the spring and summer potato and tomato crops in 1947. Therefore, it probably will not be necessary to spray or dust to control this disease.

Further notice will be given if the future weather conditions become favorable for blight.

May 22, 1947: The weather continues unfavorable for the late blight disease to develop on potatoes and tomatoes. Rainfall at Norfolk during the two week period beginning May 8th amounted on only 33 hundredths of an inch. This is considerably less than is necessary for late blight. In 1938 and 1946 when late

[5] ANONYMOUS. POTATOES. LATE BLIGHT CAUSED BY *PHYTOPHTHORA INFESTANS*. U. S. Bur. Plant Indus., Plant Dis. Rptr. 1: 12, 105. 1917. [Processed.]

[6] ———— LATE BLIGHT CAUSED BY *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 26: 8. 1923. [Processed.]

[7] COOK, H. T. DEVELOPMENT OF TOMATO AND POTATO LATE BLIGHT. U. S. Bur. Plant Indus., Plant Dis. Survey, Late Blight Warning Service, Nos. 12, 14, 15, and 17. 1947. [Processed.]

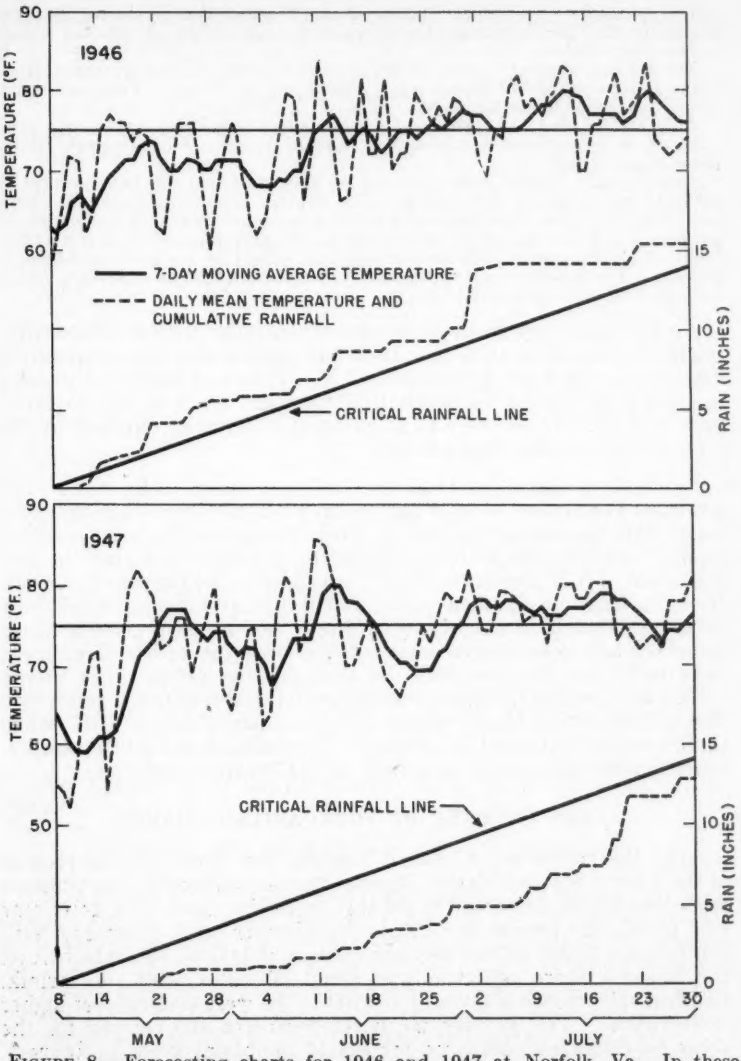

FIGURE 8.—Forecasting charts for 1946 and 1947 at Norfolk, Va. In these charts, the cumulative rainfall is plotted at daily intervals, and the temperature is plotted as the daily mean and the 7-day moving average.

blight was important, more than 2½ inches of rain fell during the same two week period.

Under the present weather conditions, it is not necessary to spray or dust potatoes or tomatoes for late blight control.

May 29, 1947: The weather conditions are still unfavorable for the late blight disease to develop on potatoes and tomatoes. The 91 hundredths of an inch of

rain recorded by the Norfolk Station of the Weather Bureau during the week beginning May 22nd benefitted the potato and tomato crops but was not enough to favor late blight.

The five-day weather forecast issued May 27th indicated above normal temperatures for the period and about ½ to 1 inch of rain on Friday. These conditions will also be unfavorable for blight.

Since the weather has been unfavorable for late blight during the first three weeks of the critical period, it is unlikely that the disease will be important during the summer of 1947.

June 5, 1947: There is very little chance that late blight will be important on potatoes and tomatoes this season. The weather conditions during the week beginning May 29th have continued to be unfavorable. Only 16 hundredths of an inch of rain was recorded by the Norfolk Weather Bureau office during that week. This amount is considerably less than would be required for blight to develop. The five-day weather forecast issued June 3rd indicates only 3 tenths of an inch of rain for the five-day period.

No forecasts were made after June 5, since the critical period when blight developed in 1938 and 1946 had passed and the temperature during the next week was about 75° F. Traces of blight did develop in a few fields during the last half of June and again during the latter part of July. These were local outbreaks and were checked by the higher temperatures that followed.

Forecasting charts for 1946 and 1947 are shown in figure 8. The graph for 1947 is not in exact agreement with the forecasts as the chart starts with the actual rainfall on May 8, whereas the daily weather report, issued on the morning of May 8, but actually giving the precipitation of 0.21 inches on May 7, was used in making the forecasts. Both the daily mean temperature and the 7-day average of the daily mean are shown in the graphs. The 7-day average gives a much smoother and more easily analyzed line than the widely fluctuating daily mean and also indicates the average temperature much better. The two graphs in figure 8 show that there was much more rain during 1946, when blight caused serious damage, than in 1947, when there were only traces of the disease. They also show that the temperature tended to be higher generally in 1947 than in 1946.

AREA COVERED BY FORECASTING CHARTS

Since the forecasts for eastern Virginia were based on the records of the United States Weather Bureau Station at Norfolk, the question arose how big an area could be reliably served by them. To determine this point, the forecasting charts for Norfolk were compared with similar ones based on the weather records at Onley, Va., which is on the Eastern Shore peninsula and about 60 miles north of Norfolk, and Roanoke, which is about 300 miles to the west and 900 feet higher in elevation. The average mean temperatures and rainfall for the three localities are compared for the 15 nonblight years in figure 9, and for the blight year 1946 in figure 10. The temperature and rainfall lines are remarkably similar for the three localities. The most significant difference is that the temperatures at Onley and Roanoke average from 2° to 3° lower than at Norfolk, It would appear from these charts that the forecasts made on the Norfolk records would be reasonably accurate for at least the eastern part of Virginia and fairly accurate for the Roanoke area, provided allowance was made for

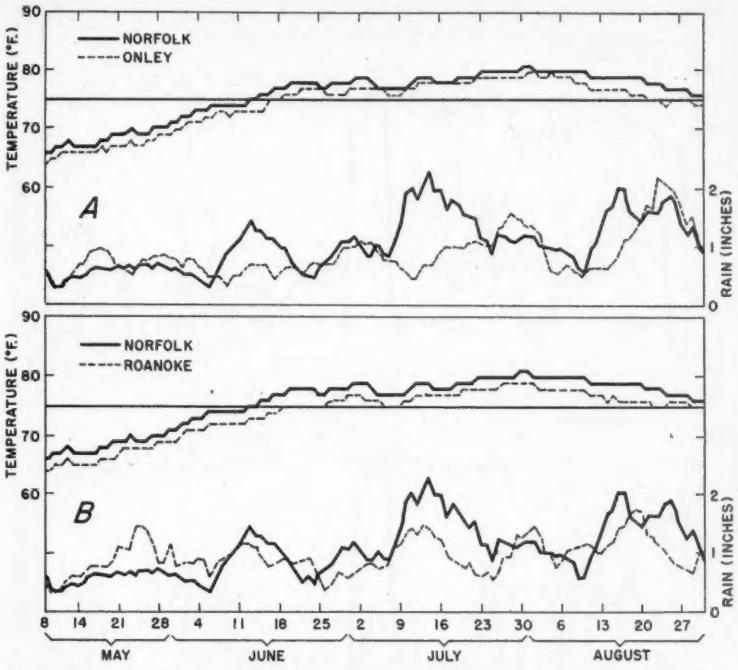

FIGURE 9.—Seven-day moving charts comparing average rainfall and temperature of the 15 nonblight years at Norfolk and Onley (*A*) and Norfolk and Roanoke (*B*).

lower temperatures as the distance to the north or the elevation increased.

COMPARISON OF THE 1946 AND 1947 SEASONS

The weather for the 1946 and 1947 potato and tomato growing seasons was compared for Norfolk, Onley, and Roanoke, Va.; Salisbury, Md.; and Dover, Del. The purpose was to see how the seasons had differed and to correlate these differences with the date of occurrence and severity of blight. (Salisbury is approximately 45 miles north of Onley, and Dover is 55 miles north of Salisbury.) These comparisons are shown in the 7-day moving charts in figure 11.

The chart for Norfolk shows that late blight in 1946 was correlated with the heavy rainfall in May. There was less rain during that period in 1947, and the blight did not develop until a period of moderately heavy rain and low temperature after the middle of June. The disease did not cause serious damage in 1947 because it started late and was checked by high temperatures about the end of June. A second outbreak of blight developed on tomatoes during a cool, rainy period the last half of July in 1947. Although generally distributed,

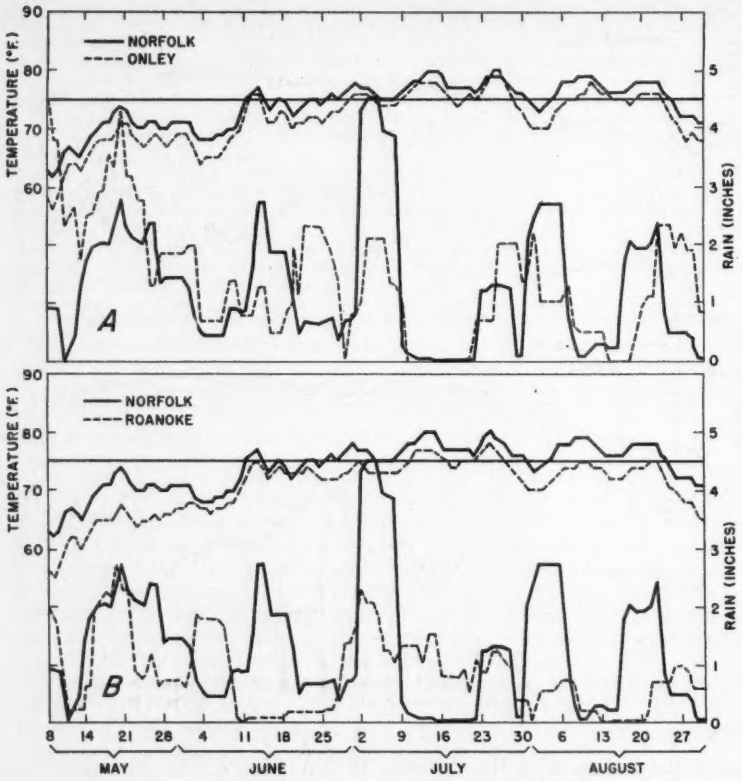

FIGURE 10.—Seven-day moving charts comparing average rainfall and temperature of the blight year 1946 at Norfolk and Onley (*A*) and Norfolk and Roanoke (*B*).

the disease caused damage in only a few isolated fields and disappeared early in August when the weather became dry and hot.

The contrast between the two seasons was even more striking at Onley than at Norfolk. Rainfall was very heavy in May and early June in 1946, and the late-blight epithytotic started about May 23. Very little rain fell during the same period in 1947, and no blight was found in the potato or tomato crop in the southern part of the Eastern Shore peninsula. An epiphytotic of short duration did develop in a small section of the Shore about 15 miles north of Onley during a rainy, cool period the last week in July. This epiphytotic was checked by high temperatures the first week of August.

The first report of late blight in the Roanoke area in 1946 was on May 27.[8] This was correlated with the heavy rains during the last half of May. The same period in 1947 was relatively dry, and no

[8] FENNE, S. B. LATE BLIGHT ON POTATO IN WESTERN VIRGINIA. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 164: 283. 1946. [Processed.]

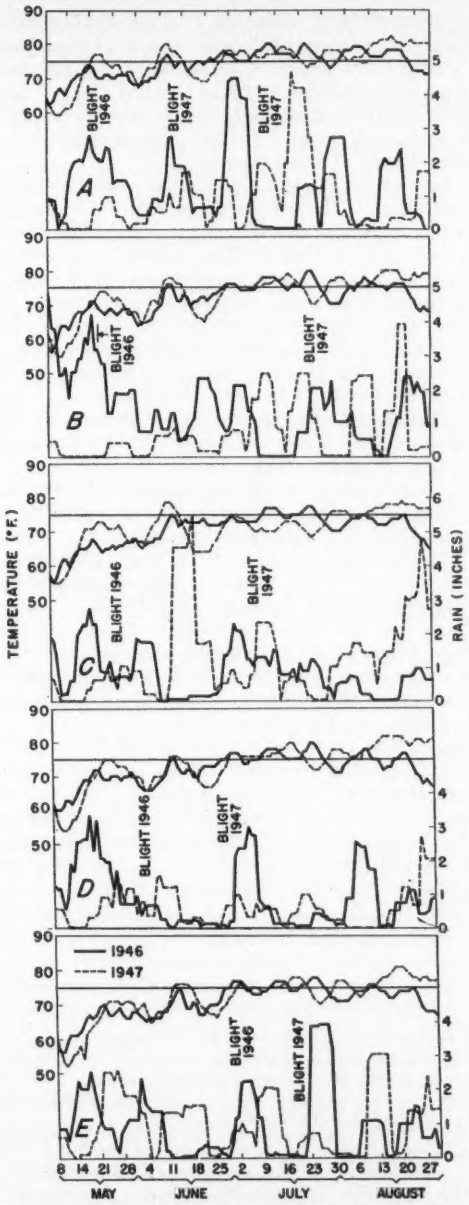

FIGURE 11.—Comparison of the rainfall and temperature of the blight year 1946 with the nonblight year 1947 at (*A*) Norfolk, (*B*) Onley, (*C*) Roanoke, (*D*) Salisbury, (*E*) Dover.

blight was reported until July 10.[8a] The 1947 outbreak coincided with moderately heavy rainfall and a low temperature. Probably late blight would have been reported from the Roanoke area about June 21 if a survey had been made at that time. Low rainfall the last two weeks of July and high temperatures in August kept the disease from spreading rapidly in 1947 even though a very rainy period occurred the last half of that month.

The chart for Salisbury, Md., shows that the 1946 and 1947 seasons were very dissimilar. There were three rainy periods in 1946, and the first report of late blight was made on June 4[9] following the heavy rains in the last half of May. The rainfall was low to moderate in the latter year, and late blight was not reported until July 2. This report is correlated with a period of low temperature during the last half of June. High temperatures during most of July and all of August prevented the disease from developing to epiphytotic proportions. In summarizing the late blight situation in 1947, Cox[10] reported that in contrast with 1946 there was no time during 1947 when late blight was truly epiphytotic throughout Maryland. He also stated that over-all losses from late blight were very light in spite of severe damage in small areas.

These two seasons were much more alike at Dover, Del., than at the other four localities studied, except for a very significant difference in temperature in August. Although late blight was found in two fields on May 29[11] in 1946, the initial wave of heavy infection did not occur until the first week in July. This outbreak coincided with a rainy period the last few days of June and first week of July and temperatures below 75° F. for about a week. Although the disease was checked temporarily by high temperatures about July 22, another rainy period early in August and moderate rains and low temperature during the rest of that month enabled the disease to spread rapidly and to destroy the crop by August 23.[12]

No late blight was reported until June 25[13] in 1947 even though the weather in May and June was about the same as in 1946. This local outbreak was still static on July 22, but the disease spread rapidly during the following week, and infection was state-wide by July 31. This rapid spread coincided with a period of moderate rainfall and low temperature from July 22 to 30. In summarizing the late-blight situation in 1947, Heuberger[13] stated:

> Very little spread and development of late blight occurred after July 31 as the weather from then on, particularly the first half of August, was generally unfavorable.

[8a] FENNE, S. B., and WINGARD, S. A. LATE BLIGHT OF TOMATOES IN WESTERN VIRGINIA. U. S. Bur. Plant Indus., Soils and Agr. Engineering, Plant Dis. Reptr. Sup. 171:209. 1947. [Processed.]

[9] COX, C. E., JEHLE, R. A., and WOODS, M. W. LATE BLIGHT OF POTATOES AND TOMATOES ON THE EASTERN SHORE OF MARYLAND. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 164: 284. 1946. [Processed.]

[10] LATE BLIGHT IN MARYLAND IN 1947. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 171: 213. 1947. [Processed.]

[11] HEUBERGER, J. W. LATE BLIGHT IN DELAWARE. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 164: 285. 1946. [Processed.]

[12] ——— LATE BLIGHT OF TOMATOES IN DELAWARE—1946. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 165: 319–321. 1946. [Processed.]

[13] LATE BLIGHT OF TOMATOES IN DELAWARE—1947. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 171: 216–217. 1947. [Processed.]

This observation is in general agreement with the chart which shows that the temperature was 75° F. or above in August except for a period of 5 days from August 5 to 10.

The comparison of the two seasons at these five locations indicates such a close correlation of the severity and time of development of late blight with the temperature and rainfall that the importance of the disease in any season appears to be more dependent on the weather conditions than on air-borne inoculum from the more southern areas.

DISCUSSION

The successful forecasting of late blight in eastern Virginia in 1947 and the study of the relation of the temperature and rainfall to the occurrence of the disease during the last 31 years at Norfolk, Va., and Charleston, S. C., indicate that it is possible to predict the probable importance of this disease with better than 80 percent accuracy in those areas.

The forecasts are for epiphytotics that affect the whole areas and do not apply to minor or local outbreaks of the disease. Even when most of an area is entirely free of the disease, local outbreaks may occur in individual fields if they are on low land, surrounded by high windbreaks or have been in the path of a succession of local showers. Also minor outbreaks may affect a considerable proportion of the acreage but not cause serious damage, because the weather conditions favorable for blight are of short duration.

The basic feature of the forecasting method described in this paper is that the predictions are made from charts of the temperature and rainfall instead of from field surveys for the presence of the disease or a general impression of the weather. The presence of the disease in fields where the environmental conditions are especially favorable for late blight does not necessarily mean that an epiphytotic is starting, and yet it may be so alarming that general spraying or dusting would be advised. A general impression of the weather is not accurate enough to serve as a basis for plant-disease-control recommendations. The weather of the last few days or extremes of temperature or rainfall make it difficult to recognize what the actual average weather has been.

The weather data from which the forecasting charts are prepared are the temperature and rainfall records of the United States Weather Bureau observers. These data are available for many localities and usually cover a long period of years. Although the development of late blight may be correlated with high humidity, relative humidity records are not so generally available or complete as rainfall records and therefore are not so practical for use in forecasting.

The basic principle in analyzing and charting the weather data is to express the average conditions instead of daily variations. In these studies, a unit of 7 days was found to give a good indication of the relation of the weather to late blight. Lines on the charts based on the 7-day units were much smoother and more easily interpreted than were the widely fluctuating lines based on daily records. No attempt was made to use totals or averages of calendar weeks or months since these periods do not necessarily coincide with the time of development or duration of an epiphytotic. The 7-day moving charts were useful

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in determining the critical period and in comparing the weather of different years or different localities. The cumulative rainfall chart was better for the actual forecasting. Both types of charts should be used simultaneously in border-line years.

Information on the past occurrence of late blight was obtained from the Plant Disease Reporter and Supplements and from the experiment station files. In many cases this information is not so complete as could be desired. Frequently an outbreak of late blight was reported but was not followed by reports on the subsequent severity or duration of the disease. Late blight may have gone unreported in some years because of lack of a survey by a pathologist interested in that crop. The more detailed information about late blight which is being collected by the Late Blight Warning Service should be especially valuable for future studies.

Although the forecasting system described in this paper appears to work in the coastal areas for the potatoes and tomatoes planted in the spring and maturing in the summer, it may need considerable modification to adapt it to winter crops, such as are grown in Florida, crops grown at higher elevations where dews are more of a factor, and in the more northern States where the crops mature in the fall.

SUMMARY

Routine spraying or dusting is not economically practical for late-blight control in many areas. A method for forecasting the probable importance of the disease would make it unnecessary to apply fungicides unless the disease threatened to cause serious damage.

A forecasting method developed for eastern Virginia was used successfully in 1947. The same system was found to be adaptable to the Charleston, S. C., area.

The forecasts were based on charts prepared from an analysis of temperature and rainfall in relation to the occurrence of late blight in the 17-year period 1930–46.

Predictions based on these charts would have been correct 84 percent of the time in the 31-year period 1917–47 at Norfolk, and 81 percent of the time during the same period at Charleston. The error would have been in the years when spraying was recommended but the disease failed to reach epiphytotic proportions.

The charts for Onley, 60 miles north of Norfolk, and Roanoke, 300 miles to the west, show remarkably similar trends in temperature and rainfall and indicate that the forecasts may apply to a fairly large area.

Comparison of the charts for 1946 and 1947 at Norfolk, Onley, and Roanoke, Va., and for Salisbury, Md. and Dover, Del., show a close correlation of the time of appearance and severity of late blight with the temperature and rainfall of the two seasons.

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INVESTIGATION OF ROTENONE AND BENZENE HEXACHLORIDE DUSTS FOR THE CONTROL OF INSECT ECTOPARASITES ON SHEEP[1]

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INTRODUCTION

The best time to treat sheep in Wisconsin for ectoparasites is immediately after shearing. For this purpose, dusts are more desirable than either dips or sprays since shearing frequently occurs during cold, early spring weather. Matthysse [2] reported effective control of sheep ked, *Melophagus ovinus* (L.) on feeder lambs with a rotenone dust (approximately 0.5-percent rotenone content) applied by means of a power duster. Dicke, et al [3] further demonstrated that a 1-percent rotenone dust gave a good, practical control of ked on unshorn sheep. Applications were made at the rate of 4 ounces per mature animal, using a simple hand-plunger duster. At a later date, preliminary trials indicated that benzene hexachloride was also a potentially effective insecticidal dust for application to sheep.

Several workers, however, have demonstrated the possibility that meat from animals dusted or sprayed with benzene hexachloride may be tainted. Hixson and Muma [4] first reported an off-flavor and odor in eggs exposed in a chicken house previously sprayed with a 5-percent benzene hexachloride suspension (0.25-percent gamma isomer content). During later trials these workers [5] reported off-flavor and odor in the flesh of chickens sprayed directly with a 0.25-percent gamma isomer benzene hexachloride suspension, or when housed in sheds previously sprayed with this insecticide. Grummer, et al [6] reported off-flavor contamination of pork muscle and fat following benzene hexachloride treatments of hogs 24 hours previous to slaughter. No contamination was observed following treatments 10 days or more before slaughter.

Sheep infested with ked, *M. ovinus*, and a species of chewing louse, *Trichodectes ovis* (L.), were therefore dusted during April and May to determine the comparative efficacy of rotenone and benzene hexa-

[1] Received for publication September 2, 1948.

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chloride as insecticides. Additional sheep were dusted with benzene hexachloride at intervals before slaughter to determine potential hazards of meat contamination.

INSECTICIDE TRIALS

PROCEDURE

A lot of 10 Shropshire ewes and 7 lambs infested with ked and lice were selected for the insecticide studies. These were divided into 3 lots and isolated in separate pens throughout the experiment. One lot was dusted with benzene hexachloride containing 1 percent of the gamma isomer, and a second lot with a 1-percent rotenone dust containing 2 percent of a No. 20 motor oil conditioner. A third lot was untreated, serving as a control. All animals were held during treatment, and applications were made with a rotary hand duster at the rate of 4 ounces of dust per ewe and 1 ounce per lamb. All but one check animal were unshorn at the time of treatment.

Infestations were determined prior to treatment and at weekly intervals following dusting for a period of 5 weeks. Relative populations of ked were made by parting the fleece and taking actual adult counts over one side of the body for ewes, and adult and pupal counts over the entire body for lambs. At the conclusion of the experiment, all ewes were sheared and counts were made of keds on the body and throughout the shorn fleece. Relative populations of *T. ovis* were not determined, and only the presence or apparent absence of lice was observed.

RESULTS

Weekly counts for ked on treated and untreated animals are summarized in table 1. Initial infestations of sheep ked were effectively controlled by both rotenone at a 1-percent concentration, and benzene

TABLE 1.—*Ked control with benzene hexachloride and rotenone dusts*

Flock No.[1]	Treatment	Initial infestation		Infestation in—									
				7 days		13 days		20 days		29 days		34 days[2]	
		Adults	Pupae	Adults	Pupae	Adults	Pupae	Adults	Pupae	Adults	Pupae	Adults	Pupae
Ewe 890	Benzene hexachloride dust containing 1 percent gamma isomer	22	41	0	32	0	----	0	----	0	----	0	----
Ewe 892		5	16	0	17	[3] 1	----	0	----	0	----	0	----
Lamb 887		4	0	0	0	0	0	[3] 1	0	0	0	2	0
Ewe 894		29	19	0	33	[3] 1	----	0	----	0	----	0	----
Ewe 898		78	25	[3] 3	34	[3] 2	----	[3] 2	----	0	----	0	----
Ewe 893	1 percent rotenone dust with 2 percent oil conditioner	34	17	0	17	[3] 2	----	0	----	0	----	0	----
Lamb 883		----	----	----	----	----	----	0	0	0	0	0	0
Ewe 895		6	2	[3] 1	8	0	----	0	----	0	----	0	----
Lamb 885		0	0	0	0	0	0	0	0	0	0	0	0
Lamb 886		0	0	0	0	0	0	0	0	0	0	0	0
Ewe 896		20	23	0	20	0	----	0	----	0	----	0	----
Ewe 897	Control untreated	33	11	33	19	15	----	5	----	5	----	13	----
Ewe 899		24	17	15	18	30	----	14	----	17	----	76	----
Lamb 884		----	----	19	0	101	3	121	11	176	20	210	13
Ewe 900[4]		1	0	4	0	6	----	1	----	2	----	2	----
Lamb 888		0	0	8	0	25	1	113	10	160	42	99	91
Lamb 889		0	0	3	0	35	3	48	0	44	2	70	34

[1] Lamb numbers are listed immediately below that of parent ewe. Lamb No. 887 was 5 days of age and numbers 885, 886, 888, and 889 were 1 day old at time of treatment. Lambs 884 and 883 were untreated since they were born 1 week and 2 weeks, respectively, after flock treatment.

[2] Counts on ewes were made over entire body and throughout shorn fleece.

[3] Keds recently emerged from pupae.

[4] Ewe shorn prior to test.

hexachloride at a concentration of 1-percent gamma isomer. Neither insecticide appeared to be effective against the pupae, although both indicated residual effectiveness beyond the normal pupal incubation period. Keds were observed on two sheep for the first 2 weeks after treatment with rotenone, although these were all recently emerged from pupae. For benzene hexacholride, in addition to recently emerged insects, mature adults were observed on one ewe (No. 898) a week after treatment and on a lamb (No. 887) at the close of the experiment. Rotenone, therefore, appeared to be somewhat more effective in killing the emerging insects. Population counts on the check ewes generally decreased during the test period. On all untreated lambs, however, populations increased rapidly.

No lice were found on any of the ewes following application of either rotenone or benzene hexachloride, although all animals were examined carefully at weekly intervals. Populations on the untreated ewes, however, remained constant throughout the experiment. Infestations of lice were not observed on any of the treated or untreated lambs.

PALATABILITY TESTS

PROCEDURE

Three lots of sheep were dusted with benzene hexachloride (1-percent gamma isomer concentration) at intervals of 1 month, 2 weeks, and 10 days before slaughter. A fourth untreated lot served as a check. Each lot comprised one yearling ewe and one wether lamb, all of which were shorn before dusting. All lots were isolated in separate pens throughout the experiment. Applications were made by means of a rotary hand duster at the average rate of 4 ounces of dust per animal.

All lambs and ewes were slaughtered on the same day and special care was taken to avoid carcasses coming in close contact with each other. The carcasses were chilled and stored at a temperature of 34° F. for 72 hours prior to cutting. Short loins were used as samples for testing. These were boned out and rolled without removing the fell. The average weight of each cut was about 2½ pounds before roasting.

The roasts were cooked in individual pans at 350° F. until their internal temperature reached 158° F. Each loin was then scored by a panel of judges according to a modification of the differential taste test suggested by Bengtsson and Helm.[7] By this method, the scorer tastes samples of which some are duplicates. In order to demonstrate differences in palatability, these samples must be correctly distinguished and matched. In addition, the judges were required to describe any objectionable odor or flavor detected.

Samples of fat and muscle tested were as follows:

Sample 1: Yearling ewe; untreated check.
Sample 2: Wether lamb; untreated check.
Sample 3: Wether lamb; dusted 10 days prior to slaughter.
Sample 4: Wether lamb; dusted 2 weeks prior to slaughter.
Sample 5: Wether lamb; dusted 1 month prior to slaughter.

To prevent tasting fatigue, these samples were tested on 2 different days, with a panel of 14 judges during the first day (test series A,

[7] Bengtsson, K. and Helm, E. Principles of Taste Testing. Wallerstein Lab. Commun. 9 (28): 171. 1946.

table 2) and 10 during the second day (test series B). During the first test period, sample 3 from a lamb treated 10 days prior to slaughter was tested in duplicate against sample 1, an untreated check. On the second day, samples 4 and 5 from animals treated 2 weeks and 1 month, respectively, were tested against both untreated checks (samples 1 and 2).

RESULTS

A summary of results is given in Table 2. During the first day of testing (test series A) only 5 out of 14 judges correctly matched duplicate samples of fat and muscle from lambs treated with benzene hexachloride 10 days prior to slaughter (samples 3A and 3B). The remaining 9 judges matched one or the other of the treated duplicate samples with the untreated check (sample 1). Only 5 judges out of 10 on the second day (test series B) correctly matched the treated samples (5A and 5B, 4 and 5A, or 4 and 5B). An equal number matched a treated sample with an untreated control (1 and 4, 1 and 5a, or 2 and 5B).

No disagreeable or unusual odor for either treated or check samples was noted during cooking. In the first series, only 2 judges out of 14 reported an unusual odor for the treated samples. Slight off-flavor in this series was reported by 6 judges, of which 2 of the samples designated were untreated checks. In the second test series, as many untreated check samples as treated were considered by the judges as having appreciable off-flavor or as being least desirable. Much of the off-flavor or odor noted, however, may have been confused with a flavor or odor typical for mutton or lamb, especially in view of the inconsistencies reported in matching the samples tested.

TABLE 2.—*Results of palatability tests by differentiation of cooked samples from treated and untreated animals*

Sample combinations reported [1]	Number of judges checking combination as duplicate	
	Test series A	Test series B
Sample 1 (untreated) + Sample 3A (treated, 10 days)	4	
Sample 1 (untreated) + Sample 3B (treated, 10 days)	5	
Sample 3A + Sample 3B (treated, 10 days)	5	
Sample 1 (untreated) + Sample 4 (treated, 2 weeks)		1
Sample 1 (untreated) + Sample 5A (treated, 1 month)		2
Sample 2 (untreated) + Sample 5B (treated, 1 month)		2
Sample 5A + Sample 5B (treated, 1 month)		1
Sample 4 (treated, 2 weeks) + Sample 5A (treated, 1 month)		2
Sample 4 (treated, 2 weeks) + Sample 5B (treated, 1 month)		2
Total	14	10

[1] Sample numbers with letter designations indicate duplicated samples; i. e., 3A and 3B.

SUMMARY AND CONCLUSIONS

Sheep infested with ked, *Melophagus ovinus*, and the chewing louse, *Trichodectes ovis*, were dusted prior to shearing with benzene hexachloride containing 1 percent of the gamma isomer and with 1-percent rotenone. Both insecticides provided complete control for lice, and an effective initial control for ked over a 34-day observation period. Rotenone, however, appeared to be more effective than benzene hexachloride in preventing reinfestation with ked emerging from pupae.

Shorn sheep were dusted with benzene hexachloride at a 10-day, 2-week or 1-month interval prior to slaughter to determine the effect of tainting on palatability of fat and muscle. Preliminary taste tests evaluated by matching treated with untreated samples failed to show that taints in meat from animals given a single dust application were more pronounced than different flavors resulting from variations in quality.

INDUCED VARIABILITY IN PHOMA LINGAM[1]

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INTRODUCTION

Recently Pound (*5*)[2] described a species of *Phoma* on seed plants of cabbage (*Brassica oleracea* var. *capitata L.*) in the important seed-producing area of the Pacific Northwest. This pathogen differed slightly in pathogenicity and cultural characters from strains of *Phoma lingam* (Fr.) Desm. that incite blackleg in the eastern part of this country. However, it was considered a variant of *P. lingam* because it fell within the range of natural variability found for that species. No evidence of seed transmission of the pathogen in cabbage of this Puget Sound strain was presented but it was pointed out that such a possibility should not be overlooked. Evidence was presented to show that seed-pod infection of rutabaga (*Brassica Napobrassica* Mill.) would occur under suitable conditions.

In the summer of 1947 a severe epidemic of cabbage blackleg occurred in Wisconsin and in several other areas of the United States. This infection was traced to Puget Sound seed of the 1946 harvest (*6*). The disease produced in the field from this infected seed was typical of cabbage blackleg in type and severity, but fungus isolates obtained from the seed were of two types. Some resembled the Puget Sound strain described by Pound in culture and pathogenicity while others were typical of eastern strains. Occasionally, isolates were obtained which resembled the Puget Sound strain in culture but eastern strains in pathogenicity and vice versa. It was important, therefore, to determine whether this sudden development of seed transmission on Puget Sound seed resulted from increased activity of the Puget Sound strain in the production of mutants typical of those occurring in Eastern States and capable of being transmitted in seed.

One way to obtain this information, it was decided, would be to make an extensive study of the induced cultural and pathogenic variability of the pathogen to see if mutants could be obtained that were similar to strains isolated from the infected seed lots. This paper is a report of such a study.

In view of the success that has been obtained in inducing mutations in two very stable strains of *Penicillium* by ultraviolet irradiation (*2*) and by the action of the nitrogen-mustard gas (methyl-bis (beta-

[1] Received for publication September 2, 1948. Appreciation is expressed to Eugene Herrling for making the photographs used as illustrations for this paper and to Lloyd Wilson for assisting with the nitrogen-mustard gas experiments discussed herein.

[2] Italic numbers in parentheses refer to Literature Cited, p. 587.

chloroethyl) amine) (*7*), it was thought that these two agents offered the best means available to induce variability in the Puget Sound strain. Although the sulfur-mustard gas (bis (beta-chloroethyl) sulfide) has been reported to be very efficient in inducing mutation in *Drosophila* and *Neurospora* (*1*, *4*), the nitrogen-mustard gas was selected since it has an effect similar to that of sulfur-mustards and is easier to handle in the laboratory (*3*, *7*). In certain experiments other strains of *Phoma lingam* other than the Puget Sound isolate and other methods of inducing variability were used for comparison.

MATERIALS AND METHODS

The source of strains PS40, S1, S39, Iowa II, and W11 of *Phoma lingam* used in the study was transfers of the single-spore cultures described by Pound (*5*). PS40 was the strain from the Puget Sound section; S1 and S39 were from Oregon; and Iowa II and W11 were originally from Iowa and Wisconsin, respectively.

The nitrogen-mustard treatment consisted of suspending the spores in freshly prepared aqueous bicarbonate buffered solution of methyl-bis (beta-chloroethyl) amine as reported by Stahmann and Stauffer (*7*). The monochromatic ultraviolet light treatment consisted of irradiating a suspension of spores with a narrow band of ultraviolet radiation at 2,750 Å (angstroms) as reported by Stahmann and Stauffer (*7*). The polychromatic irradiation was carried out by exposing single-spore cultures in petri plates to a Westinghouse Sterilamp (WL 782–30) radiation held at a distance of 9 cm. above the cultures. This is an open type mercury-quartz lamp which emits 80 to 90 percent of its energy at 2,536 Å.

Agar medium from the same source was used when comparing mutants. It consisted of 200 gm. of potatoes, 20 gm. of dextrose, and 20 gm. of agar per liter. A 25-ml. sample was used per petri dish.

Following treatment with nitrogen mustard or ultraviolet radiation the treated spores were seeded to petri dishes containing potato-dextrose agar and when germinated, individual spores were transferred to potato-dextrose agar slants. When the slant cultures had matured, the percentage of mutants was calculated. Adequate controls of untreated spores served as the basis of mutant diagnosis. Mutants were transferred successively 3 times as a test of stability. Representative cultures of each mutant type obtained were selected and saved.

Two methods of testing the pathogenicity of representative mutants were used. One was to soak cabbage seed in a spore or finely macerated mycelium suspension for 48 hours before planting. Inoculations of older cabbage and Chinese cabbage (*Brassica pekinensis* Rupr.) plants were made by spraying the suspensions on the leaves and incubating the plants in a moist chamber for 48 hours. The cabbage leaves were rubbed with cabbage juice containing a small amount of powdered carborundum to remove some of the bloom and thus enhance wetting of the leaves.

EXPERIMENTAL RESULTS

VARIABILITY PRODUCED BY NITROGEN-MUSTARD GAS

Tables 1, 2, and 3 give the results of treatments with nitrogen-mustard gas on two strains of *Phoma lingam*, S1 and PS40. The

data obtained show that the nitrogen-mustard exerted a toxic action which continued throughout the treating period. Although the nitrogen-mustard was very effective in producing morphological mutants, it is evident that the percentage of mutants of the survivors reached a maximum and then declined. The action of the nitrogen-mustard is nonselective as it kills the mutant and nonmutant spores alike. This is indicated by the decline in the percentage of mutants obtained under long exposures.

The results of treating the S1 strain are given in table 1.

TABLE 1.—*Induction of mutants in the S1 strain of Phoma lingam by nitrogen-mustard (methyl-bis (B-chloroethyl) amine)*

[Concentration of nitrogen-mustard was 0.005 mM. per ml.]

Length of treatment (minutes)	Spores surviving		Mutants among survivors [2] (percent)	Length of treatment (minutes)	Spores surviving		Mutants among survivors [2] (percent)
	Number [1]	Percent			Number [1]	Percent	
0	250,000	100.00	2.0	16	6,800	2.72	31.8
1	85,000	34.00	24.0	32	4,460	1.78	39.8
2	41,620	16.65	27.0	64	2,760	1.10	29.8
4	20,600	8.24	20.9	128	1,800	.72	13.9
8	14,360	5.74	26.0	256	100	.04	([3])

[1] Values given are calculated from the average number of spores surviving in each of 5 plates multiplied by the amount of dilution each sample received.
[2] Calculated from a random sample of 100 single spores from colonies surviving treatments, from 0 minute to 8 minutes, inclusive; from the rest of the treatments all of the survivors were saved.
[3] The number of survivors was too small to ascertain the percentage of mutants.

TABLE 2.—*Induction of mutants in the PS40 strain of Phoma lingam by nitrogen-mustard (methyl-bis (B-chloroethyl) amine)—Experiment 1*

[Concentrations of nitrogen-mustard were 0.01, 0.02, and 0.04 mM. per ml.]

Length of treatment (minutes)	Spores surviving		Mutants among survivors [2] (percent)	Length of treatment (minutes)	Spores surviving		Mutants among survivors [2] (percent)
	Number [1]	Percent			Number [1]	Percent	
0.01 mM.							
0	9,170	100.00	0	16	220	2.40	36.4
1	2,320	25.29	12.1	32	140	1.53	21.4
2	1,680	17.88	14.6	64	90	.98	22.2
4	660	7.19	15.2	128	70	.76	14.3
8	380	4.14	26.3	256	20	.22	25.0
0.02 mM.							
0	9,170	100.00	0	4	220	2.40	27.3
1	1,040	11.34	3.8	8–256	0	0	0
2	920	10.03	8.7				
0.04 mM.							
0	9,170	100.00	0	4	60	.65	66.7
1	480	5.23	8.3	8–256	0	0	0
2	200	2.18	40.0				

[1] Values given are calculated from the average number of spores surviving in each of 5 plates multiplied by the amount of dilution each sample received.
[2] Calculated from a random sample of 100 single spores from the 0-minute treatment. All of the survivors were saved from the rest of the treatments.

With this strain a maximum was reached in the production of mutants in the 32-minute treatment and was followed by a decline to no mutants in the 256-minute treatment. At this point the treatment was 99.6 percent lethal so that the actual number of survivors was too low (an average of only one colony per plate) to obtain an estimate of the percentage of mutants in the survivors.

TABLE 3.—*Induction of mutants in the PS40 strain of Phoma lingam by nitrogen-mustard (methyl-bis (B-chloroethyl) amine)—Experiment 2*

[Concentrations of nitrogen-mustard were 0.01 and 0.015 mM. per ml.]

Length of treatment (minutes)	Spores surviving		Mutants among survivors (percent)	Length of treatment (minutes)	Spores surviving		Mutants among survivors (percent)
	Number [1]	Percent			Number [1]	Percent	
0.010 MM.							
0	122,720	100.00	[2] 0	16	2,900	2.36	15.0
1	56,920	46.38	1.1	32	4,980	4.06	12.4
2	22,680	18.48	9.0	64	1,670	1.36	28.1
4	12,980	10.58	12.0	128	2,350	1.91	28.4
8	5,900	4.81	7.4	256	1,330	1.08	23.2
0.015 MM.							
0	122,720	100.00	[3] 0	16	920	.75	57.8
1	28,540	23.25	6.1	32	400	.33	50.0
2	5,640	4.59	29.6	64	120	.10	45.4
4	3,320	2.71	37.8	128	58	.05	40.0
8	1,220	.99	56.7	256	25	.02	[4]

[1] Values given are calculated from the average number of spores surviving in each of 5 plates multiplied by the amount of dilution each sample received.
[2] Calculated from a random sample of 100 single spores from the 0-minute through the 8-minute treatments. All of the survivors were saved from the rest of the treatments.
[3] Calculated from a random sample of 100 single spores from the 0-minute to the 4-minute treatments. All of the survivors were saved from the rest of the treatments.
[4] See footnote 3, table 1.

The effect of increasing the concentration of the nitrogen mustard on the PS40 strain can be obtained by an examination of tables 2 and 3. These tables show that the percentage of survival decreased more and more rapidly as the concentration increased, and with increase in concentration there was a corresponding decrease in the length of treatment required to reach a maximum percentage of mutants. Unless a fairly strong concentration was used, there was always a very small percentage of survivors left at the end of the treatment period. The 0.02- and 0.04-mM. (millemol) per ml. concentrations (table 2) were extremely toxic to the spores.

It may be pointed out that although both concentrations effected complete killing sometime before the 8 minute sample was taken, the 0.02-mM. per ml. concentration was much less severe than the 0.04-mM. concentration. Table 3 shows that a much higher number of mutants was obtained by increasing the concentration of the nitrogen mustard from 0.010 to 0.015 mM. This was decidedly the most optimal of all treatments used for inducing variability in this strain.

Description of Mutants

Range of variation in cultural characters

Many mutants produced were apparently identical, and, from such groups, representatives were chosen for further study. To compare the representative nitrogen-mustard-induced mutants of the PS40 strain, each was transferred to petri dishes containing potato-dextrose agar and a description record of the cultural characteristics was taken. Although most of the isolates were macroscopically different from the untreated PS40 strain and from each other, they could be placed in arbitrary groups according to their pigmentation and growth rate. Figure 1 shows some representative strains of the PS40 nitrogen-mustard-induced mutants growing on potato-dextrose agar. All cultures were photographed at the same age. Although numerous mycelial transfers were made of the isolates, very little change was noted from the original cultural appearance.

The mutants exhibited a wide range of variation in cultural characters. A complete range of growth rates with corresponding degrees of staling was encountered. Some strains made a growth of only 10 mm. in diameter in 3 weeks and then staled almost completely. Others grew somewhat faster, reaching a diameter of about 35 mm. in 3 weeks, before complete staling occurred. The growth of many of the isolates was slow at first, with only moderate staling apparent. Eventually, however, that is in 3 or 4 weeks, they filled the 90-mm. petri dishes; others were capable of reaching a maximum of 90 mm. diameter in 7 or 8 days with no evidence of staling.

In general the very fast cultures had a very regular mat outline, and the slower growing cultures tended to have very irregular mat outlines. The irregular mat outlines varied from uneven lobes of mycelium protruding from the edges of the colonies to the dendritic, feathery margins of some isolates.

The topography of the colonies was also very variable. In some isolates the colonies were very flat, while in others a considerable elevation was noticed which was usually accompanied by considerable convolution and folding of the mycelium. In a few of the strains the major portion of the colony was submerged, only the very center of the colony being raised above the surface of the agar. The amount and texture of the aerial mycelium is of interest in that for some strains it was scant while in others it was very abundant. The texture of the aerial mycelium ranged from very fine to very coarse.

The color usually was white at first but varied considerably with age, becoming gray, grayish-brown, brown, reddish-tan, light tan, yellow, cream, or pink or remaining white. With age also in some of the isolates the aerial mycelium darkened in color and in some cases they apparently collapsed either in whole or in parts of the colony. The color of the colonies not exhibiting any noticeable aerial mycelium varied from flesh-colored to dark brown. The color of the mycelium from the lower side of the petri dish also varied widely between mutants.

Mutants differed markedly in their ability to sporulate. Some strains produced no pycnidia; others produced pycnidia only sparingly, and still other strains produced pycnidia abundantly.

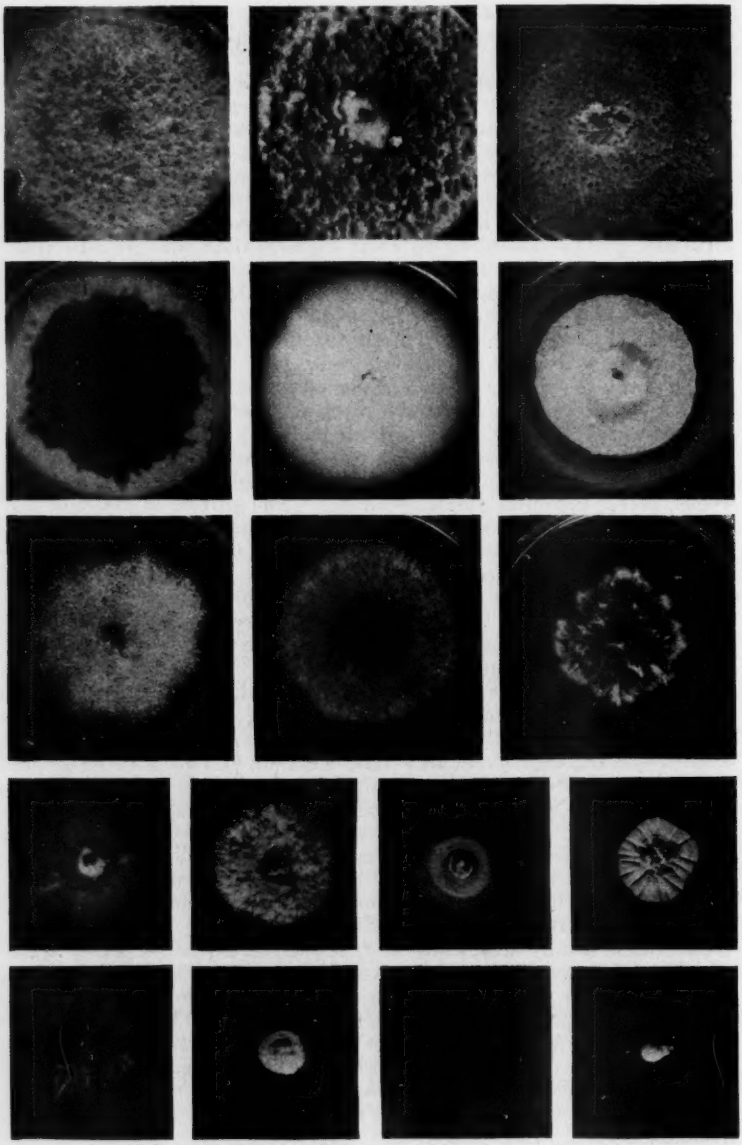

FIGURE 1.—Representative mutants of strain PS40 induced by nitrogen-mustard treatments compared with the parental strain, shown in the upper left-hand corner.

The size of the pycnidia produced ranged from very small to quite large. The distribution of the pycnidia varied from those produced singly and scattered evenly over the surface of the colonies to those produced in clumps. Many of the pycnidial lines produced so much aerial mycelium that the pycnidia were completely obscured.

When the PS40 strain of *Phoma lingam* was grown at room temperature it produced a yellow, water-soluble pigment which diffused into the agar medium. At relatively high temperatures of about 32° C. this yellow color became plum red. The nitrogen-mustard-induced mutants of this strain were capable of producing a range of pigmentation from yellow to yellowish brown to a deep plum-red color when grown at room temperature. A few of the strains produced very little pigment but all eventually did so with age.

The nitrogen-mustard-induced mutants of the S1 strain were not studied in as much detail as were the mutants of PS40. No PS40-type mutants were obtained from S1, although a fairly wide range in type of mutants was found. Although mutants from S1 were usually faster growing forms, some slower growing strains were obtained. The mat outline of the colonies varied in regularity. The aerial mycelium varied from very scant to very abundant. In many of these mutants the aerial mycelium occurred in irregular patches or cottony balls. Most of the mutants obtained retained their ability to sporulate quite heavily, although some were recovered which produced few pycnidia but many sclerotium-like bodies scattered over the surface of the agar. Only in a few cases were any mutants obtained in which pycnidial production was completely inhibited. All of the mutant strains produced a black pigment beneath the mycelial mat.

PATHOGENICITY TESTS

Pound (*5*) developed a method by which the eastern virulent strains can easily be differentiated from the western mildly pathogenic strains. Within 2 weeks after cabbage seed previously soaked in a spore or mycelial suspension have emerged after planting, the seedlings develop cotyledonary lesions and severe damping-off. The characteristic lesions of the PS40 strain appear 3 to 5 days earlier than those of the eastern type (e. g., Iowa II), and are usually marginal and chocolate brown from the beginning and enlarge slowly; only a few, relatively large pycnidia are produced.

Cotyledonary lesions produced by the Iowa II strain of *Phoma lingam* appear as shrunken dark-green areas, which enlarge rapidly and quickly produce many pycnidia before the lesion becomes dark. Figure 2 shows the western (PS40) and the eastern (Iowa II) types of cotyledonary lesions. On older cabbage leaves there is also a differential method of separating the eastern and western types of cultures. Figure 3 shows the spreading, sporulating lesions produced by the virulent eastern type culture of Iowa II and the black, necrotic flecks typical of the PS40 strain. The results of the inoculations are shown in table 4.

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TABLE 4.—*Results of inoculating seeds and leaves of cabbage and leaves of Chinese cabbage with representative nitrogen-mustard-induced mutants of the PS40 strain of Phoma lingam*

Isolate No.	Inoculum (mycelium or spores)	Virulence on cabbage Cotyledons [1]	Disease index [2] on leaves of—		Isolate No.	Inoculum (mycelium or spores)	Virulence on cabbage Cotyledons [1]	Disease index [2] on leaves of—	
			Cabbage	Chinese cabbage				Cabbage	Chinese cabbage
41	M	++++	100.0	75.0	22	S	++	50.0	0.0
18	M	+++	91.7	94.4	34	S	++	50.0	41.7
36	M	+++	91.7	66.7	88	S	+++	50.0	22.2
4	S	+++	88.9	58.3	32	M	++	50.0	25.0
31	S	+++	83.3	36.1	42	M	+	49.2	50.0
71	S	+++	80.6	38.9	75	S	+++	47.2	11.1
3	S	+++	77.8	44.5	52	S	+++	47.2	19.4
2	S	+++	77.8	33.3	61	S	+++	44.5	27.8
12	S	+++	77.8	22.3	86	M	+++	44.5	36.1
82	M	++	75.0	36.1	67	S	+++	44.4	16.7
87	S	+++	75.0	36.1	28	M	+	41.7	0.0
39	M	+++	72.2	50.0	58	S	+	41.7	41.7
14	M	+++	72.2	25.0	21	S	+	41.7	8.3
8	M	+	72.2	25.0	77	S	++	41.7	38.9
1	M	+	72.2	0.0	56	M	+	41.7	50.0
5	M	+	72.2	0.0	84	M	+++	38.9	33.3
16	M	+	72.2	0.0	50	S	+++	38.9	30.6
29	M	+	72.2	0.0	55	S	+	38.9	41.7
13	M	+	69.4	0.0	72	S	++	36.1	47.2
37	M	+	66.7	75.0	45	M	+	36.1	27.8
69	S	+++	66.7	22.2	59	M	+	33.3	25.0
9	M	+	63.9	38.9	66	S	+++	33.3	25.0
38	M	+	63.9	27.8	60	S	+++	33.3	25.0
63	S	+++	63.9	50.0	64	S	++	33.3	39.8
43	S	+++	63.9	38.9	46	M	+	33.3	8.3
70	S	++++	63.9	33.3	76	M	++	30.5	33.3
26	M	+	61.1	28.0	78	S	+	30.5	5.5
10	S	++	61.1	8.3	80	S	+	27.8	22.2
11	S	++	61.1	0.0	85	S	++	27.8	13.9
17	S	+	61.1	0.0	47	S	++	27.8	25.0
31	M	+	61.1	0.0	6	M	+	27.8	25.0
19	S	+++	61.1	38.9	73	M	+	25.0	30.5
48	S	+++	58.8	25.0	79	S	+	25.0	0.0
25	M	+	58.3	0.0	33	S	+	25.0	30.5
15	M	+	55.6	16.7	34	S	++	25.0	2.8
40	M	+	55.6	58.3	74	M	+	25.0	38.9
69	S	+++	55.6	33.3	49	S	+	25.0	33.3
20	S	+++	55.6	0.0	48	S	+	25.0	22.2
35	M	++	55.6	33.3	81	M	+	22.7	25.0
53	M	+	52.8	25.0	83	S	++	22.2	13.9
65	S	+++	52.8	16.7	57	M	+	19.4	22.2
62	S	+	52.8	25.0	54	S	0	8.3	2.8
30	M	+	52.8	50.0	PS40 control	S	++	47.4	33.5
7	S	++	52.8	8.3	Iowa II control	S	++++	97.2	58.3
27	M	+	52.8	0.0	L. S. D. (19:1)			19.3	15.9
24	M	+	50.0	11.1					

[1] 0=no infection; +=slight; ++=moderate; +++=severe; ++++=very severe.
[2] Calculated by rating each of 3 leaves of 3 plants; 0=no infection; 25=slight; 50=moderate; 75=severe; 100=dead.

In no instance, either with cabbage seedlings or with larger cabbage and Chinese cabbage plants did any of the 88 mutants produce symptoms of the Iowa II type. When symptoms were produced they were always identical in type with the PS40 parent strain.

An examination of table 4 will reveal that marked differences in virulence existed among the mutants. Leaves of cabbage and Chinese cabbage inoculated with the PS40 parent strain gave an average disease index of 47.4 and 33.5, respectively. Numerous replications of the parent strain were made and no significant difference existed between them. In the case of cabbage leaves, 21 of the 88 mutants were more virulent than the parent strain while 15 were less virulent. On Chinese cabbage, however, only 11 were more virulent while 29

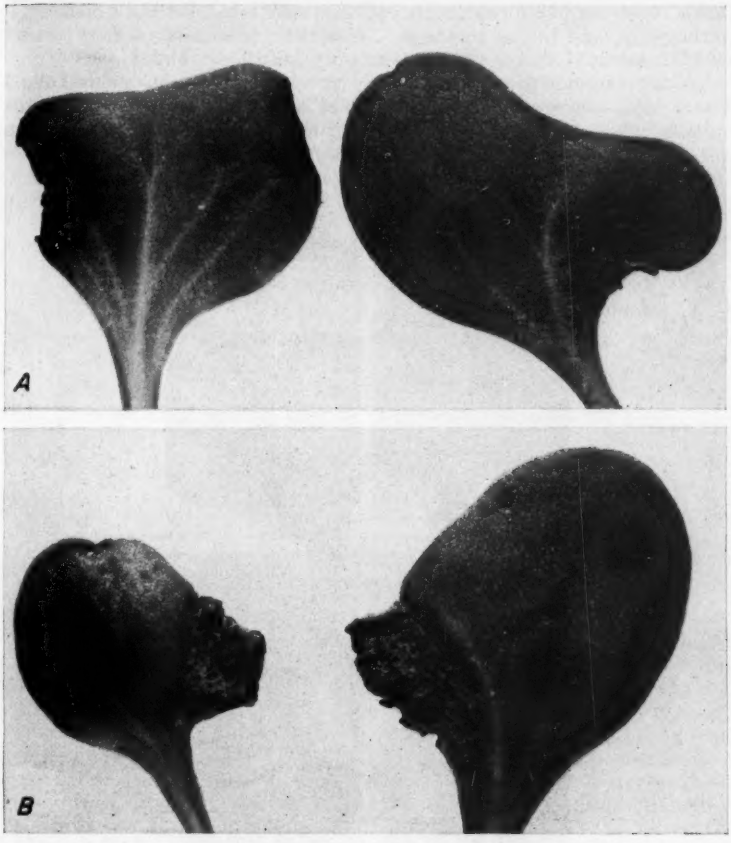

FIGURE 2.—Cotyledonary lesions produced on cabbage seedlings by the western strain PS40 (A), and by the eastern strain Iowa II (B), of *Phoma lingam*. Note the heavy sporulation of Iowa II and the almost complete absence of pycnidia in PS40.

were less virulent than the check. It is notable that several of the mutants failed to infect Chinese cabbage leaves although ideal conditions for infection were provided.

A second series of inoculations were made in which the isolates showing higher virulence and those showing lower virulence on Chinese cabbage were retested. Results similar to those shown in table 4 were obtained. Recovery inoculations from affected tissue to potato-dextrose-agar were made and in each case isolates recovered closely agreed culturally with those forms used as inocula. Thus, in passage through host tissue, the mutants gave no evidence of reversion to the parent type.

A study of table 4 will also reveal that there appears to be no correlation between virulence on cabbage and Chinese cabbage. Some

forms were highly virulent on cabbage and weakly virulent or nonpathogenic on Chinese cabbage. However, few isolates were severe on Chinese cabbage and they were only mild on cabbage.

To ascertain whether there was any correlation between cultural characters and virulence, the isolates were arbitrarily divided into four cultural groups according to their growth rate and pigmentation on potato-dextrose-agar. These data further revealed the lack of positive correlation between virulence on cabbage and Chinese cabbage.

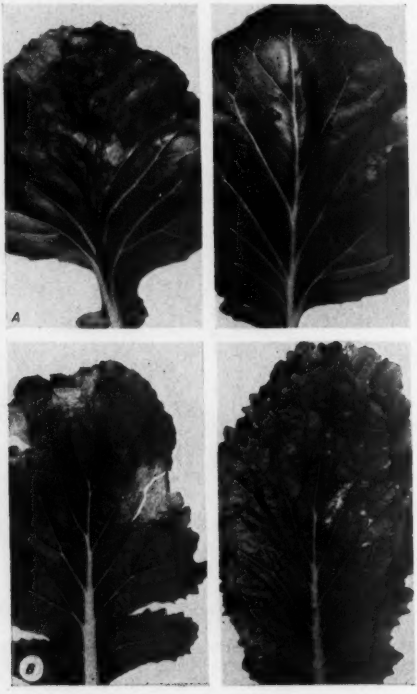

FIGURE 3.—*A*, Symptoms produced on cabbage leaves by the eastern strain Iowa II (left), and by the western strain PS40 (right), of *Phoma lingam*. *B*, the effect of the two strains on leaves of Chinese cabbage.

In some cases there appeared to be a definite negative correlation although this was not always the case.

VARIABILITY PRODUCED BY ULTRAVIOLET IRRADIATION

MONOCHROMATIC IRRADIATION

Table 5 gives the results of treating a spore suspension of the PS40 strain with a narrow band of ultraviolet radiation of 2750 Å. The table shows that this wave length exerted a considerable toxic effect which continued throughout the treatment period. The percentage of mutations of these survivors reached a maximum and then declined.

That the ultraviolet radiation is also nonselective is indicated by this subsequent decline in the number of mutants.

Figure 4, based on statistics given in tables 3 and 5, shows graphically two essential differences between the results of 0.015 mM. per ml. nitrogen-mustard and the 2750 Å. ultraviolet radiation treatments.

The curves in figure 4 and figure 5 show that both treatments are effective in reducing the spore populations.

TABLE 5.—*Induction of mutants in the PS40 strain of Phoma lingam by monochromatic ultraviolet radiation*

Length of treatment (minutes)	Spores surviving		Mutants among survivors[2] (percent)
	Number[1]	Percent	
0	230,000	100.00	0
1	193,000	83.91	0
2	182,200	79.22	4.0
4	177,500	77.17	4.0
8	127,500	55.43	5.0
12	126,800	55.13	16.2
16	71,400	31.04	16.3
20	49,500	21.52	20.0
24	33,900	14.74	40.4
28	10,600	4.61	26.0
32	7,500	3.26	28.6
36	3,100	1.52	25.0

[1] Values given are calculated by multiplying the average number of spores surviving in each of 5 plates by the amount of dilution each sample received.

[2] Calculated from a random sample of 100 single spores from the 0-minute through the 24-minute treatment from the rest of the treatments all of the survivors were saved.

Figure 5 shows the effect of treating two very closely related strains, S1 and S39, with 0.005 mM. per ml. of nitrogen-mustard and 2750 Å. ultraviolet radiation, respectively. Since no data were taken on the rate of mutation in the S39 strain, no comparisons can be made between the mutation rates.

The nitrogen-mustard treatment at 0.015 mM. per ml. concentration appears to be more effective in bringing about mutations in the PS40 strain than the ultraviolet treatment at 2750 Å, when the mutation rate is based on the spores surviving treatment.

No essential differences were noted in the two treatments with respect to the morphological types of mutants produced.

POLYCHROMATIC IRRADIATION

Table 6 summarizes the results of exposing 2-week old single spore cultures of the S1 and W11 strains of *Phoma lingam* to polychromatic ultraviolet radiation. The treated cultures were incubated for 2 weeks, and any sectors arising were then isolated. The data in the table show that this radiation had a marked effect upon the colonies but that the length of treatment had little effect. The sectors in the controls may be explained by the long incubation period. In some control plates no sectors occurred, however. Mycelial transfers from the sectors of the S1 strain were found to be stable through several transfers. Similar transfers were made from the apparently normal portions of the colonies and these were found to resemble closely the untreated parent strain.

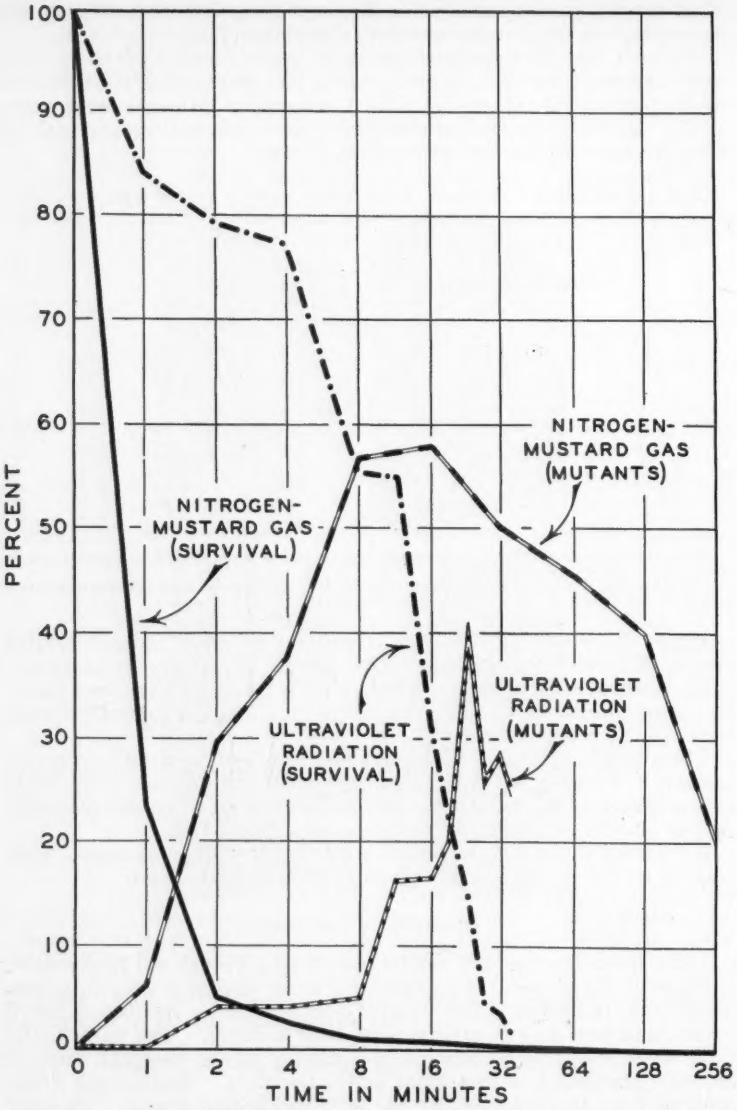

FIGURE 4.—Curves showing the differences between the percentage survival of spores and the mutation rates from 0.015 mM. per ml. of nitrogen-mustard gas treatment (methyl-bis (B–chloroethyl) amine) and 2750 Å. ultraviolet radiation of the PS40 strain of *Phoma lingam*.

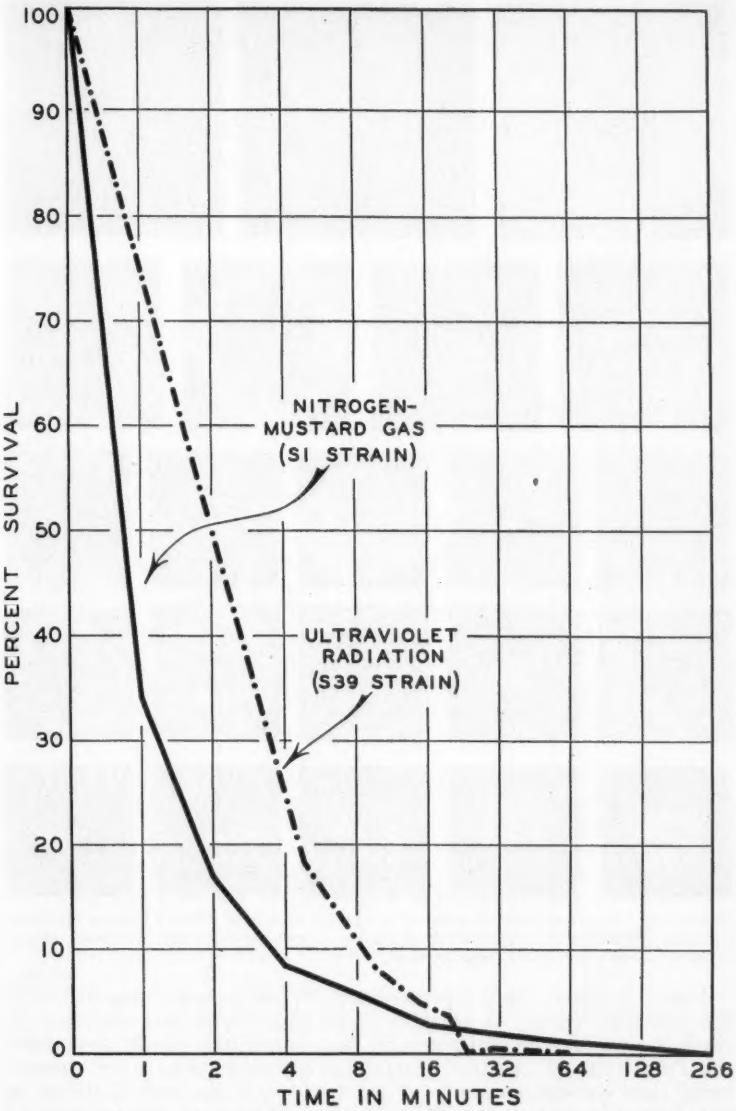

FIGURE 5.—Curves showing the effect on spore survival of ultraviolet radiation on the S39 strain and of nitrogen-mustard gas (methyl-bis (B–chloroethyl) amine) treatment on the S1 strain of *Phoma lingam*.

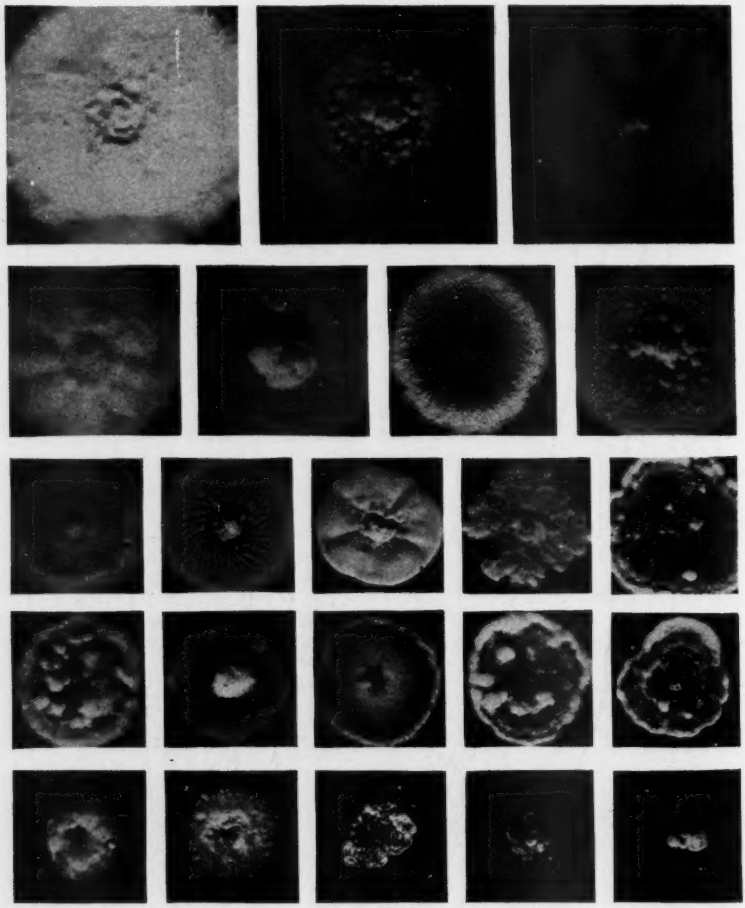

FIGURE 6.—Representative mutants of the S1 strain of *Phoma lingam* obtained from polychromatic ultraviolet radiation compared with the parental strain, shown in the lower left-hand corner.

Figure 6 shows some representative S1 mutations compared with the parental strain. A variable array of mutants was obtained including some fast-growing strains but none that closely resembled the PS40 type of culture. Some slow-growing forms were encountered that consisted mainly of piled up pycnidia with little or no aerial mycelium present. The aerial mycelium ranged from scant to abundant and in many cases was collected in irregular patches or cottony balls. Various color differences in the aerial mycelium were observed. Pycnidia production ranged from none to abundant and when pycnidia were present they were usually quite small and evenly distributed over the surface of the mycelium except in the very center

of some colonies and in those strains in which the pycnidia were produced in concentric bands. These forms were stable through several transfers. Black pigment was always produced beneath the mycelial mats.

TABLE 6.—*Induction of mutants in single-spore cultures in the S1 and W11 strains of Phoma lingam by exposure to polychromatic ultraviolet radiation*

Length of treatment (minutes)	Average number of sectors per plate [1]		Length of treatment (minutes)	Average number of sectors per plate [1]	
	S1	W11		S1	W11
0	2.1	2.4	8	7.8	6.0
2	8.2	6.6	16	6.6	5.3
4	7.6	6.6	32	7.2	6.3

[1] 18 plates were used as controls and 5 plates for each time treatment for strain S1. 8 plates were used as controls and 3 for each time treatment for strain W11.

VARIABILITY PRODUCED BY UNFAVORABLE TEMPERATURES

Table 7 gives the results of exposing the PS40, S1, and W11 strains of *Phoma lingam* to a series of temperatures from 4° to 36° C. over a relatively long period. After single, germinated spores were transferred to potato-dextrose agar plates, they were placed immediately at the various temperatures. The table shows that relatively high temperatures cause these strains to be more unstable. The data obtained indicated that the spores were unable to withstand the 36° C. treatment, for in no case did colonies develop.

A complete range of growth rates was found for the range of temperatures used, with the PS40 strain growing considerably faster in all cases. The optimum temperature for PS40 was 24° C. while 20° C. was optimum for the S1 and W11 strains. At 32° C. strains S1 and W11 were greatly inhibited and the PS40 strain was slightly so. The mutants obtained were stable and were not different in type from those obtained by other treatments, but much fewer mutant types were obtained.

No PS40 type mutant was obtained in either the S1 or W11 strain. The PS40 mutants that were obtained by this treatment were all fast-growing and differed from the parent in the color of the aerial mycelium and the degree of sporulation. The mutants of strains S1 and W11 differed from the parental strains in growth rates, amount and color of the aerial mycelium, and in degree of sporulation.

TABLE 7.—*Induction of mutants in single spore cultures in the PS40, S1, and W11 strains of Phoma lingam by exposure to various temperatures over a 3-week period*

Treatment (° C.)	Average number of sectors per plate [1]			Treatment (° C.)	Average number of sectors per plate [1]		
	PS40	S1	W11		PS40	S1	W11
4	0	0	0	24	0	0.7	0
8	0	.2	0	28	.3	1.0	.4
12	0	.6	0	32	.5	2.0	3.2
16	0	0	0	36	[2]		
20	.1	.8	0				

[1] 10 plates were used per treatment with PS40 and 5 plates per treatment with S1 and W11.
[2] All cultures killed by 36° C. treatment.

The effect on the S1 strain of short exposures to high temperatures is shown in table 8. The spore suspension was treated in 1-ml. portions held in small capillary tubes that were submerged in a constant-temperature water bath. At the end of 10 minutes the capillary tubes were plunged into cold water. The spore suspension was pipetted into petri dishes containing potato-dextrose agar. It can be seen from the data that as the population decreased the average number of mutants increased. It is notable that a much lower percentage of mutants was produced by this treatment than by nitrogen-mustard treatment and by ultraviolet radiation.

TABLE 8.—*Induction of mutants in the S1 strain of Phoma lingam by exposure of spores to high temperatures in a water bath for 10-minute periods*

Temperature (° C.)	Average number of colonies per plate [1]	Average number of mutants per plate
Control	300.0	0
47	150.0	0
48	45.0	.6
49	18.0	1.2
50	.4	2.0
51–55	0	0

[1] With each treatment 5 plates were used.

VARIABILITY PRODUCED BY UNFAVORABLE MEDIA

The combination of peptone and dextrose in three otherwise favorable media caused single-spore colonies of the S1 and W11 strains of *Phoma lingam* to become quite unstable. Numerous sectors were observed in these strains growing on water agar, potato agar, and Czapek's solution agar to which peptone plus dextrose had been added. The S1 strain was not observed to throw any sectors when either peptone or dextrose was added or when both were deleted. The W11 strain was subject to slight sectoring in potato agar, potato-dextrose agar, and potato-peptone agar but otherwise behaved like the S1 strain. A wide variation in the appearance of the colonies on the different media was observed.

DISCUSSION

The induced variants of *Phoma lingam* described herein are considered to be mutants since, through several repeated transfers, no changes were observed in the microscopic morphological characters, and, since there was no evidence of reversion in passage through host tissue. No sexual stage has been reported for this fungus and thus no means is available by which the inheritance of induced characters might be studied. The vegetative hyphal cells are multinucleate and the pycnospores are uninucleate. Since the parental strains were in all cases derived from single spores heterocaryosis would not explain the variability obtained.

There was apparently no correlation between the type of mutants produced and the method of treatment employed. This could be most easily seen in the case of the PS40 strain in which a large number of mutants were obtained by different treatments. The mutants

produced by the various concentrations of nitrogen-mustard could not be distinguished by their cultural characters from those induced by ultraviolet radiation.

In the pathogenicity tests which were made to determine the amount of pathogenic variability induced in the PS40 strain, it is significant that no mutant produced lesions characteristic of those produced by eastern type strains. However, marked changes in virulence were produced. Some of the PS40 mutants produced a disease as severe as any eastern type strain while others were unable to infect Chinese cabbage even under ideal conditions. It is realized that a disease index rating system gives only a rough quantitative measure of the severity of symptoms but it is believed that sufficient evidence is presented to show that marked differences in virulence existed among the mutants.

This study does not provide full explanation for the transmission of blackleg on cabbage seed grown in the Puget Sound area in 1946. Although no mutants were obtained from the Puget Sound strain (PS40) which gave disease symptoms identical with those of eastern strains, such an array of variability in culture and virulence was obtained that the possibility of change in nature from the mild Puget Sound type to the virulent eastern type cannot be overlooked.

SUMMARY

Induced variability of the Puget Sound strain of *Phoma lingam* recently described as occurring on cabbage seed plants has been studied in relation to a sudden development of seed transmission on Puget Sound seed. In determining if this blackleg epidemic resulted from increased activity of the Puget Sound strain a nitrogen-mustard (methyl-bis (B-chloroethyl) amine) was used to induce cultural and pathogenic variability. In certain experiments other strains and other methods of inducing variability were used. Among the other treatments employed were ultraviolet radiation, growth at unfavorable temperatures and on unfavorable media.

It appeared that the rate of mutation could be markedly increased by appropriate treatment and the nitrogen-mustard appeared to be the most effective means used by which mutations could be induced. In one experiment over 60 percent mutations occurred among surviving spores. There was apparently no correlation between the type of mutant produced and the method of treatment used.

Greenhouse inoculations were made to determine the extent of induced pathogenic variability by nitrogen-mustard. No isolate produced lesions characteristic of those produced by the eastern type strains, but evidence is presented to show that through increased virulence some of the isolates were able to produce a disease that was as severe as that produced by the eastern strains. Complete evidence was not found, however, that would clarify the sudden development of blackleg transmission on western grown seed.

The Puget Sound strain is still considered only a variant of *Phoma lingam* although it is quite distinct from eastern strains of the species in growth characters and in pathogenicity. A wide range of variability in both cultural and pathogenic characters was induced by appropriate treatment. Stable forms were obtained that resembled

the eastern type cultures in certain cultural characters and in virulence but differed in symptom type. Mutants of the PS40 strain were found that exhibited a complete range of growth rates from very fast to extremely slow. Growth types ranged from cultures that were composed mostly of submerged mycelium to those producing an abundance of aerial mycelium, the texture of which varied from very fine to very coarse.

Some of these isolates possessed virulence that was equal to that of the eastern type strains. Certain points of difference from eastern strains were maintained in that practically all of these PS40-induced mutants differed from all eastern types and from the Oregon S1 and S39 strains by the production of a water-soluble, yellow, brown or plum-red pigment in the media, and they produced different types of symptoms on cabbage seedlings grown from inoculated seed and on inoculated leaves of older cabbage plants. The distinctiveness of the PS40 strain was also indicated by the fact that none of the mutants, induced or spontaneous, from typical isolates from other parts of the country resembled it closely, although Pound (*5*) found one spontaneous mutant that resembled it closely in pathogenicity. Conversely, none of the mutants from PS40 resembled the typical isolates or their mutants.

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PHYSIOLOGY AND PATHOGENICITY OF THE CUCURBIT BLACK-ROT FUNGUS[1]

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INTRODUCTION

The black-rot fungus, *Mycosphaerella melonis* (Pass.) Chiu and J. C. Walker, was first reported in France in 1891 on leaves of a Chinese variety of cucumber (*Cucumis sativus* L.) (*21*).[3]

It was recorded the next year from Delaware on vines of watermelon (*Citrullus vulgaris* Schrad.), squash (*Cucurbita* spp.), pumpkin (*Cucurbita pepo* L.), muskmelon (*Cucumis melo* L.), and cucumber (*3*). Grossenbacher (*7*) studied the disease in the greenhouse in New York and reported that when vines were inoculated through wounds those of watermelon and muskmelon were very susceptible; those of pumpkin, squash, gourd (*Lagenaria vulgaris* Ser.), and dishcloth gourd (*Luffa cylindrica* Roem.) were only mildly affected, and those of cucumber and of bur cucumber (*Cucumis anguria* L.) were not infected. Weber (*24*) later noted wilting of leaves of young cucumber seedlings in Florida. The disease has been reported on fruits of cucumber, muskmelon, squash, pumpkin, watermelon, chayote (*Sechium edule* Schwartz), balsam pear (*Momordica charantia* L.), and Chinese preserving melon (*Bennincasa hispida* Cogn.) (*17*, *24*, *25*). Black rot has been found more often in the southern than in the northern part of the United States.

When, in 1945, vines of watermelon, muskmelon, and squash were found to be severely affected by the disease in southern Wisconsin, the present investigation was undertaken. A study of the morphology and variability of the pathogen was made and has been reported (*4*). A study of the physiology and pathogenicity of the fungus is reported in the present paper.

MATERIALS AND METHODS

In a previous paper (*4*) variability in sporulation and in cultural characters of the wild type, *As*, and of a number of naturally occurring and induced mutants was described. Most of these strains were used in one or another of the experiments described herein. They are listed with certain of their outstanding characteristics in table 1.

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[2] The authors are indebted to Eugene H. Herrling for preparation of the illustrations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 614.

TABLE 1.—*Symbols and major cultural characteristics of strains of Mycosphaerella melonis used*

Strain symbol	Sporulation [1]	Character of mycelium and sporulation on potato-dextrose agar
As	+++	Aerial mycelium white, becoming gray; submerged mycelium olive to dark olive brown.
A	+ or O	Do.
A-1	O	Aerial and submerged mycelium white; growth slow.
A-2	O	Aerial and submerged mycelium white at first, changing to slightly yellowish when old; very cottony in appearance; growth rapid.
A-2s	++	Similar to A-2 except for change in color to yellowish brown or purplish brown.
B-a	+++	Aerial mycelium scanty or none; submerged mycelium hyaline at first, becoming olive brown; fruiting bodies small and black.
B-b	+++	Original character intermediate between *B-a* and *As*, later changing to As.
B-1a	++++	Aerial mycelium none or sparse; submerged mycelium hyaline at first becoming olive brown; fruiting bodies large and dark brown.
B-1b	++++	Same as B-1a except that the fruiting bodies in the early stage of development assumed a more greenish color.
B-3	O	Aerial mycelium fluffy and velvety, purplish brown; submerged mycelium purplish brown; growth very slow.

[1] O—no sporulation; +—sparse; ++—slight; +++—moderate; ++++—profuse sporulation.

In experiments in which spore germination was involved suspensions of pycnospores were made up to a standard density. This was done by adjusting the density of the suspension to that of a suspension of a $10^{-3.7}$ concentration of barium chloride in 1-percent sulfuric acid.

Seeds were germinated in sand and the seedlings were transplanted to soil in 4-inch pots, one to several plants per pot, or they were sown directly in 4-inch pots and thinned to the desired number of plants per pot. The procedures followed in various inoculation experiments are described in connection with each experiment. At the end of certain experiments plants were placed in four arbitrary classes as follows: 0, healthy; 1, slightly diseased; 2, severely diseased; 3, killed. A disease index for each lot of plants was calculated by multiplying the number of plants in each class by the class number, dividing the sum of the products by the product of the total number of plants and the highest class number, and finally multiplying the quotient by 100.

EXPERIMENTAL RESULTS

SPORE GERMINATION

Grossenbacher (*7*) reported that pycnospores from crushed pycnidia all germinated in sterile water within 24 hours. In the present investigation it was found that germination of ascospores and pycnospores in conductivity water was nil, in distilled water it was very sparse, and in sterile tap water it varied from zero to 65 percent germination. Since germination of certain fungus spores is known to be stimulated by certain plant extracts (*26*), the effect of such extracts and of fresh plant tissue on germination of spores of this fungus was studied.

Orange pulp was crushed and filtered through a Whatman No. 42 filter paper. A portion was used fresh and the remainder was autoclaved at 15 pounds pressure for 15 minutes. A single dormant cotyledon of a watermelon seed and one of a Table Queen squash (*Cucurbita pepo* L.) seed were each macerated with 20 milliliters

of distilled water, and the extract was filtered through Whatman No. 42 filter paper and used without sterilization. Ten milliliters of distilled water and 0.2 milliliter of plant extract were mixed with 0.2 milliliter of a spore suspension made up to standard density and mounted in hanging drops on clean pyrex slides in a moist petri dish. Very small pieces of cotyledons from dormant seeds and from green cotyledons of watermelon and Table Queen squash were added to hanging drops of pycnospores and ascospores suspended in water. The percentage of germinated spores was determined after 12 hours at 24° C.

In the presence of orange extract, either fresh or autoclaved, 85 to 100 percent of both ascospores and pycnospores germinated. In the presence of fresh extract of watermelon or Table Queen squash dormant cotyledon, close to 100 percent germinated but in the autoclaved extract only 45 to 60 percent germinated. When a tiny piece of green or dormant tissue of watermelon or Table Queen squash cotyledon was present in the hanging drops, spore germination approached 100 percent and germ tubes tended to grow toward the tissue.

Germination tests with orange extract and with the extract of dormant cotyledons of watermelon seeds were carried out at 8°, 10°, 12°, 20°, 24°, 28°, 32°, and 36° C. Mounts in sterile tap water were used as controls. From the results shown in figure 1, it is evident that the optimum temperature for pycnospore germination was between 24° and 28° when plant extracts were used as stimulants, while in sterile tap water the optimum was between 20° and 24°. In the presence of plant extracts growth of germ tubes was also best at 24° and 28°.

In view of the favorable effect of dextrose on growth of the fungus in semisolid medium (*4*), its effect together with that of sucrose on pycnospore germination was studied. The sucrose and dextrose employed were of reagent grade, but no attempt was made to remove any possible growth-promoting contaminants. Germination tests were carried out directly in pyrex petri dishes in M/1, M/2, M/4, M/8, M/16 and M/32 solutions to which the plant tissue extract was added. Mounts in distilled water were used as controls. The percentage of germination, the vigor, color, and shape of the germ tubes, and the degree of swelling of the cells were noted.

At 24 hours, it was noted that increase in molar concentration of dextrose caused an increase in the percentage of germinating spores but the total germination was low, the swelling of the spores was very slight, and the germ tubes were thin and short. When 0.2 milliliter of orange extract was added to one petri dish of each treatment observations made at the end of 48 hours showed that all pycnospores were swollen and had germinated in every petri dish. The germ tubes were longest and most vigorous in the case of orange extract in distilled water and became shorter and darker in color as the molar concentration of dextrose increased. In M/2 and M/1 solutions budlike hyphae consisting of olive-colored, short cells were formed. In petri dishes in which no orange extract was added, there was little change in the cells and the germ tubes, except that the latter had grown slightly in length.

In sucrose solutions, there was almost no germination at any concentration in the first 24 hours. When 0.2 milliliter of orange extract was added to one petri dish of each treatment, vigorous germination

occurred at the end of 48 hours in the low concentrations, and the percentage germination decreased with increase in molar concentration. Cells swelled more or less in each treatment and the length of germ tubes decreased as the molar concentration increased. The effects of sucrose solutions with orange extract and dextrose solutions without orange extract are shown in figure 2.

The fact that high germination occurred at high concentration of dextrose without the addition of orange extract and that low germination occurred at high molar concentration of sucrose in the presence of orange extract, showed that factors other than the osmotic pressure were concerned. The dextrose has been reported by others (*6*, *12*) as an essential factor in spore germination. However, it is obvious from the present data that the effect of dextrose is additive but is not very

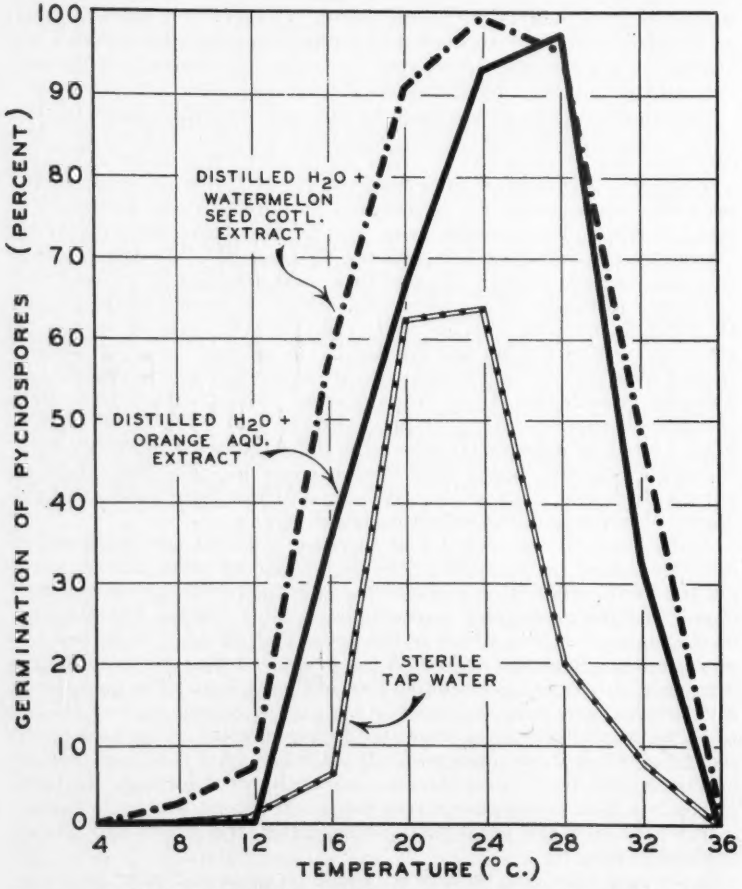

FIGURE 1.—Effect of plant extracts and of temperature on the germination of pycnospores of *M. melonis* at 24 hours.

effective without the presence of an essential stimulant. The lower availability of sucrose for growth of certain fungi has been reported elsewhere (*18*, *19*). With the present fungus, it evidently exerted an inhibitive effect and antagonized the stimulative effect of the orange extract. The inhibitive and antagonizing effects were also additive.

Duggar (*6*) noted variation in the stimulative and inhibitive effect of certain minerals. In a recent study Lin (*12*) claimed that conidia of *Glomerella cingulata* (Ston.) Spauld. and Schrenk require carbon, magnesium, nitrogen, and phosphorus for germination. Since the soluble carbohydrate, supplied in the above experiment, did not insure good germination of the pycnospores, the effect of lack of certain

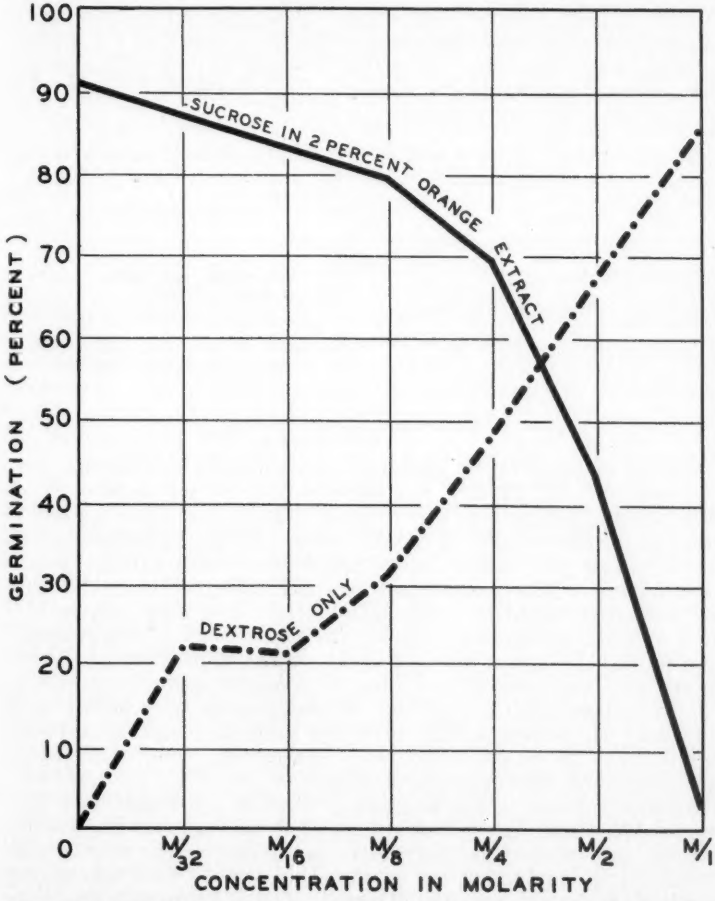

FIGURE 2.—Effect of sucrose in 2-percent orange extract and of dextrose without orange extract on germination of pycnospores. No germination occurred when sucrose alone was used. When 2-percent orange extract was added to dextrose solutions complete and vigorous germination occurred throughout.

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minerals was considered. In the present experiment a modification of Duggar's solution [4] which contained all essential minerals, was used with and without a stimulant. Two plates containing 5 milliliters of nutrient solution with 0.2 milliliter of pycnospores suspension at standard density were compared with two plates containing the same nutrient to which 2 percent of orange extract was added. Two percent of orange extract was used as a standard control. All treatments were kept at 24° C. for 24 hours when the percentage of germination as well as the vigor and length of germ tubes were recorded. A summary of the data is shown in table 2. Although 45 percent of the spores germinated without orange extract the swelling of spores before germination was slight and the germ tubes were very thin. When germ tubes in nutrient solution with orange extract and in 2-percent orange extract in distilled water are compared, it is evident that the nutrient solution had an inhibitive rather than a stimulative effect on germination. Thus the mineral salts present in the solution were not essential for germination of pycnospores.

TABLE 2.—*Nutrient solution in relation to pycnospore germination of M. mclonis*

Medium	Germination	Description of germ tubes
	Percent	
Modified Duggar's solution	45	Spores swollen only slightly; germ tubes short and no branches.
2-percent orange extract in modified Duggar's solution.	73	Spores swollen moderately; germ tubes longer than above and occasionally branched.
2-percent orange extract in distilled water	98	Spores swollen normally; germ tubes vigorous, long and profusely branched.

TOXICITY OF MINERAL SALTS

Control of black rot by spraying with bordeaux mixture has been suggested (*13*, *23*, *24*, *25*), but there is little or no evidence of an experimental approach to this phase of the disease. The toxicity to pycnospores was studied. The salts selected for the experiments were copper sulfate, zinc sulfate, aluminum sulfate, mercuric chloride, and silver nitrate. The appropriate concentrations necessary for each salt were determined by preliminary trials. For each salt an M/1 stock solution was made and the desired concentrations were prepared by dilution. Dilutions were set up in geometric progression. After the dilution was made 0.2 milliliter of standard spore suspension of a *B–1a* culture and 0.2 milliliter of orange juice were added to 10 milliliters of solution so that each test solution contained approximately 30,000 spores per milliliter. Conductivity water, with orange juice added, was used as a control solution.

Methods for testing the fungistatic effect of a toxicant have been recommended and discussed elsewhere (*1*, *9*, *11*, *14*, *15*). The various external and internal factors which may influence the results were considered in conducting the germination tests. Since the pycnospores of the fungus germinated equally well in drops on glass slides

[4] KnO_3 0.1 M (10 milliliters); KH_2PO_4 0.05 M (10 milliliters); $MgSO_4$ 0.01 M (10 milliliters); ferric sulfate 0.000002 M (0.5 milliliter); dextrose 0.25 M (25 milliliters).

and in a shallow layer of the solution in the bottom of a petri dish (*11*), both methods were used. Nine-centimeter pyrex petri dishes were cleansed with cleaning solution, washed with tap water, rinsed thrice in distilled water, and dried in dust-proof desiccators. Five milliliters of the inoculated test solution was pipetted into each of two petri dishes, which were tipped gently until the spore suspension was evenly distributed in a thin but continuous layer. Observations were made with a microscope by focusing on the bottom of the dish, since most of the pycnospores sank promptly to the bottom. In the case of drop-on-slide tests, U-shaped glass rods were used as supporters for the slides in 90-millimeter pyrex petri dishes lined with clean blotting paper moistened with the same solution used in the respective tests. Pyrex slides were cleaned as in the case of the petri dishes and one slide containing three drops of the prepared spore suspension was placed in each dish. For each concentration of solution two slides were prepared, giving a total of six drops for each test. The test slides and dishes were incubated at 24° to 28° C. Each experiment was set up at about 4 p. m. and the counts were finished before 11 a. m. the next morning. In the case of each salt, six successive experiments were run by the drop-on-slide method. These were supplemented by petri-dish tests with copper sulfate, zinc sulfate, and aluminum sulfate.

The criterion adopted for positive germination was the presence of a germ tube visible under the microscope. Those spores that swelled but did not produce a germ tube were considered nongerminative. The total number of spores and the percentage of germinating spores were determined in a standard area of 54 square millimeters marked with India ink on a cover glass placed in the ocular of the microscope. In order to facilitate the counting, this area was again divided into 6 small squares. The counting was done with the aid of a hand counter. The average percentage germination of spores in six samples taken from six drops of the same concentration, or from two petri dishes of the same treatment was recorded. The observed percentage of germination in a given solution was subtracted from the percentage germination of the control, the difference was multiplied by 100, and the product was divided by the percentage germination in the control to secure the Abbot correction for mortality.

The method used for measuring the LD50 and LD95 follows those suggested by Bliss (*2*) and later by Wilcoxon and McCallan (*27*) and by Dimond et al. (*5*). The percentage of inhibition of the germination of viable spores in a certain concentration of solution as calculated above was first transformed into probits (*2*), and then plotted against the logarithm of the corresponding molarity of the solution. The LD50 and LD95 were taken directly from the curves.

The results recorded in table 3 were from spores germinated directly in pyrex petri dishes. The order of the relative toxicity of the tested salts, except $Al_2(SO_4)_3$, measured in both LD50 and LD95 was $AgNO_3$ $HgCl_2$ $CuSO_4$ $ZnSO_4$. Using $CuSO_4$ set at unity, as a basis for comparison, $AgNO_3$ was 100 (LD50) or 82 (LD95) times more toxic than $CuSO_4$ in terms of molarity. Likewise $HgCl_2$ was 21 (LD50) or 30 (LD95) times as toxic. $ZnSO_4$ was only 0.33 (LD50) or 0.47 (LD95) times that of $CuSO_4$ $Al_2(SO_4)_3$ was nearly equal i ntoxicity to $ZnSO_4$ at the LD50 level but its toxicity dropped to almost one-third that of $ZnSO_4$ at the LD95 level.

TABLE 3.—*Toxicity of some mineral salts on the germination of pycnospores of Mycosphaerella melonis when germinated directly in petri dishes*

Salt	Concentration	Germination	Inhibition	LD50	LD95
	mMx10^{-3}	*Percent*	*Percent*	*mMx*10^{-3}	*mMx*10^{-3}
$CuSO_4$	0	95.8			
	2.0	90.7	5.3		
	4.0	87.6	8.5	7.6	15.8
	8.0	57.6	39.9		
	10.0	41.6	56.7		
	12.0	9.5	90.6		
$ZnSO_4$	0	95.3			
	7.0	91.6	3.9		
	14.0	86.1	8.3	23.0	33.9
	21.0	67.0	28.5		
	28.0	16.0	80.4		
	42.0	1.1	99.0		
$Al_2(SO_4)_3$	0	95.9			
	4.5	91.0	3.4		
	7.5	87.7	8.5	22.9	55.0
	10.5	81.1	15.5		
	15.0	72.7	25.1		
	22.5	49.1	48.9		
	37.5	17.7	81.8		
$HgCl_2$	0	96.6			
	.05	90.2	6.7		
	.14	83.9	13.1	.36	.53
	.28	76.5	24.7		
	.35	41.9	65.9		
	.50	13.8	87.5		
$AgNO_3$	0	95.3			
	.006	93.0	3.5		
	.018	91.6	3.9	.076	.130
	.060	83.5	12.4		
	.090	17.5	81.1		
	.120	3.1	96.1		

The results recorded in table 4 were obtained by drop-on-slide tests. The toxicity of $Cuco_4$ was lower than in the previous experiment, but that of $Al_2(SO_4)_3$ was slightly higher. The relative order of $CuSO_4$ and $ZnSO_4$ was the same but $Al_2(SO_4)_3$ was twice as toxic as $CuSO_4$. Because of this discrepancy, the data on toxicity of aluminum sulfate were considered to be inconclusive.

TABLE 4.—*Toxicity of some mineral salts on the germination of pycnospores of Mycosphaerella melonis when germinated in drops on slides*

Salt	Concentration	Germination	Inhibition	LD50	LD95
	mMx10^{-3}	*Percent*	*Percent*	*mMx*10^{-3}	*mMx*10^{-3}
$CuSO_4$	0	96.6			
	4.0	88.9	7.9		
	8.0	86.1	11.3		
	16.0	75.6	21.5	36.3	63.1
	32.0	57.4	40.1		
	40.0	34.9	64.1		
	48.0	21.2	78.6		
	64.0	3.5	96.4		
$ZnSO_4$	0	96.3			
	28.0	92.4	4.4		
	56.0	87.7	9.3		
	70.0	83.9	12.6	87.1	111.0
	91.0	37.4	61.1		
	98.0	17.2	82.2		
	101.0	2.8	93.5		
$Al_2(SO_4)_3$	0	97.6			
	3.0	93.3	5.2		
	6.0	86.8	9.9		
	12.0	79.8	18.4	17.4	30.9
	19.5	36.9	62.3		
	25.5	18.9	80.4		
	30.0	3.2	96.7		

The concavity of certain curves of dosage-response study of some inorganic salts was first observed by McCallan et al. (*15*). In the present experiment, almost all curves obtained showed that character (fig. 3). On probits paper, each curve consisted simply of a breaking straight line. Such breaks occurred, in general, below the LD50 level. Therefore the use of LD50 for evaluation was still reliable except for

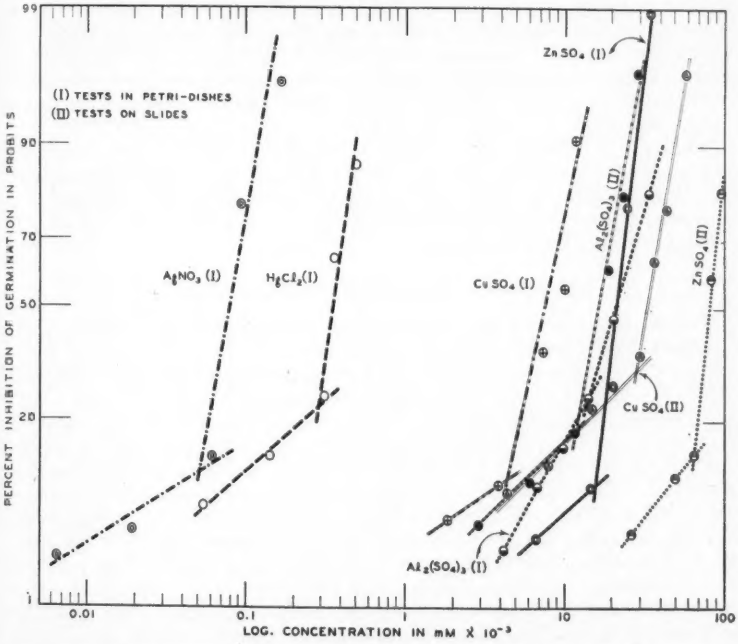

FIGURE 3.—Comparison of the toxicity of some mineral salts on the germination of pycnospores of *M. melonis* when exposed in pyrex petri dishes and in drops on slides. Each value represents the average of six experiments.

aluminum sulfate tested in petri dishes. The change of slope of these curves in the lower dosage indicates that the response of the protoplasmic substance of the pycnospores in the lower dosage was less sensitive than in higher dosages. Such a change of protoplasm-dosage response might be responsible for the slope of curves.

The advantage of germinating certain kinds of fungus spores directly in petri dishes for toxicity tests have been discussed by Lin (*11*). As preliminary trials revealed, this method seemed satisfactory for the present experiment. When Lin applied it to the toxicity of copper sulfate to the germination of conidia of *Sclerotinia fructicola* (Wint.) Rehm, he found that the toxicity of copper was several times as great as that observed by McCallan and Wilcoxon (*14*) on slides. However, he did not point out the cause of this discrepancy. Obviously no two toxicity tests are exactly alike, as already recognized by many workers. The present experiment yielded evidence

that both copper sulfate and zinc sulfate were more toxic when pycnospores were tested directly in petri dishes than when they were tested on slides. However, the difference did not hold true in the case of aluminum sulfate. It is believed that oxygen was an important factor in the discrepancy between the two methods of germination. Although oxygen shortage was insignificant when no toxicant was present, it might be expected that when certain toxicants disturbed the regular enzymic or respiratory functions of the living system of the pycnospores, an increase in need of oxygen might reveal shortage of the latter. On the other hand, aluminum might have a different toxic effect on the living system of the pycnospores, and therefore not show a difference, as copper and zinc did, between the two methods of testing germination.

Nielson (*16*) found silver salts to be effective protectants against *Phytophthora infestans* (Mont.) DBy. In this study 0.01 gamma per milliliter of silver as silver nitrate inhibited 95 percent of the germination of the pycnospores of *M. melonis* as compared with 4.3 to 19 gamma per milliliter of copper.

RELATION OF TEMPERATURE TO GROWTH

Wiant (*25*) studied the temperature relations of this fungus and reported that no growth occurred at 35° and 40° F. (1.7° and 4.4° C.), and only slight growth at 45° F. (7.2° C.), maximum growth occurred at 80° F. (26.7° C.), and no growth occurred at 95° F. (35° C.). In the present study pycnospores were seeded uniformly on potato-dextrose agar plates which were incubated at 12°, 16°, 20°, 24°, 28°, 32°, and 36° C., respectively. In every treatment seven replications were prepared. The diameters of fungus colonies were measured at intervals of 24 hours. The results are presented graphically in figure 4. The optimum lay between 20° to 24° C. The upper limit for growth was 36°, while the lower limit was somewhat below 12° C. The results were in general agreement with those of Wiant (*25*). Interestingly, the fungus seemed to have the same optimum temperature for growth as *Ascochyta pisi* Lib. and *Mycosphaerella pinodes* (Berk. & Blox.) Stone, as reported by Hare and Walker (*8*). The acceleration of the rate of growth was rather uniform and rapid at the optimum temperature. At 12° and 16° C. the growth started very slowly, but the rate of acceleration was nearly as great as at higher temperatures.

RELATION OF REACTION OF MEDIUM

The relation of hydrogen-iron concentration to growth and sporulation was studied with semisolid potato-dextrose agar and with modified Duggar's liquid medium. The former was titrated with HCl and NaOH to the following pH values: 4.0, 5.0, 6.5, 7.0, 8.5, and 9.5. Four plates for each value were prepared and seeded uniformly with the sporulating *As strain*. All plates were incubated at 24° C., and the diameters of the colonies were measured at 24-hour intervals for 4 days. Coloration and sporulation were recorded on the tenth day. The modified Duggar's solution was titrated with HCl and NaOH to the desired pH values. Fifty-milliliter aliquots of each solution were placed in 150-milliliter Erlenmeyer flasks and autoclaved at 15 pounds for 15 minutes. The pH value was determined after steri-

lization. The initial pH values after sterilization were 2.6, 4.1, 5.9, 7.1, 8.0, 8.8, and 9.8. Each flask was seeded with one drop of pycnospore suspension made from a *B–1a* culture. All flasks were incubated at 24°. Observations were made every day with special reference to sporulation. At the close of the eighth day, all cultures

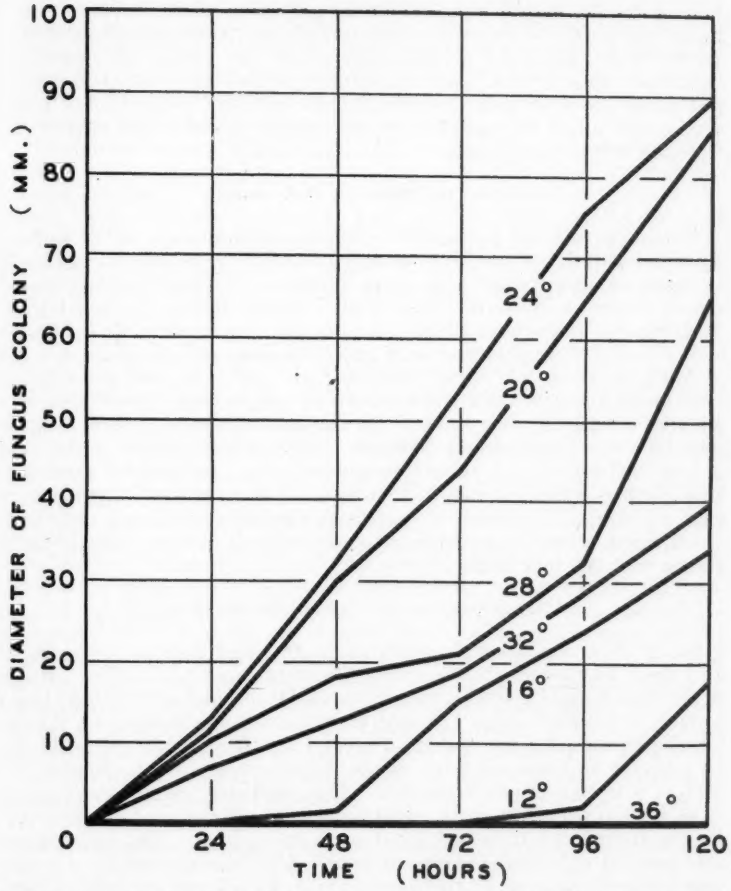

FIGURE 4—Relation of temperature to growth of *M. melonis* on potato-dextrose agar.

were filtered through previously desiccated and weighed filter papers. Mycelial mats and filter paper were then dried at 102°.

In the semisolid medium there was no significant difference in the growth rate from pH 5 to 9.5; and slight decrease occurred at pH 4.0 and 5.0. Sporulation was most abundant at pH 6.5 to 7 and was absent only at pH 9.5. The degree of coloration was correlated closely with sporulation. In the liquid medium the dry weight of mycelium

varied little within the range of pH, 5.9 to 9.8. A slight decrease in weight occurred at pH 4.1 and no growth occurred at pH 2.6. The results were similar to those obtained in the semisolid medium. The fruiting bodies appeared first at a pH of 4.1 on the third day, next at pH 5.9 on the fourth day, at pH 7.1 on the sixth day and at pH 8 to 9 on the seventh day. Variation in the abundance of fruiting bodies was not significant. The final pH value of the filtrate of each flask was usually 6.4 to 6.6. The earliest and most abundant sporulation in the semisolid medium was at pH 6.5 to 7.0. It appeared, therefore, that in the liquid medium sporulation was favored at a pH value close to neutral and that the earlier the favorable point was produced in the medium by the fungus growth, the earlier the fruiting bodies developed.

OVERWINTERING OF THE FUNGUS

Naturally infected watermelon stems bearing mature perithecia and pycnidia and dried fruiting cultures of the *As* type (*4*) on agar and on steamed watermelon stems were wrapped in cellophane and placed out of doors at Madison, Wis., from November 1945, to April 1946. Watermelon and squash stems from plants inoculated in the field with a *B–1a* culture were left in wire gauze on the surface on the ground at Madison from November 1946, to June 1947. In both seasons the perithecia and pycnidia were empty by spring but the fungus was isolated readily in every case. In the 1945 material, a new crop of pycnidia was produced by May 29. In the 1946 material, the host debris disintegrated without production of a new crop of pycnidia. The *As* type was recovered in 1946 and the *B–1a* in 1947. It appears that the fungus lives over in northern winters as dormant mycelium from which a new crop of pycnidia is produced in the spring to serve as the primary inoculum.

PATHOGENICITY AND DISEASE DEVELOPMENT

An *A* type culture was grown on a sterilized mixture of oatmeal and sand as previously described for corn-meal sand (*20*). Seeds of Hubbard squash (*Cucurbita maxima* Duchesne) and Table Queen squash (*Cucurbita pepo* L.) and Hawkesbury and Improved Kleckley Sweet watermelon, sterilized with 1:1,000 mercuric chloride for 10 minutes and washed with sterile water, were sown in furrows in each of four flats of sterilized soil. The prepared inoculum was added to each furrow in three flats, and a layer of sterilized sand was applied. In the fourth flat only sterilized sand was added. The experiment was carried out in a greenhouse at 28° C. The reduction of emergence was 26 percent in Hubbard squash, 18 percent in Table Queen squash, 15 percent in Hawkesbury watermelon, and 6 percent in Improved Kleckley Sweet watermelon. On established seedlings, spotting of cotyledons was common but no symptoms appeared on the primary leaves. Hypocotyl cankers were found only on about 16 percent of the Table Queen squash seedlings. When the nonemerged seeds were examined, it was found that some seeds were still dormant, while others had started to germinate and had been killed. Isolations from such plants yielded *A* type cultures.

Symptoms on the cotyledons varied with the species and variety. On watermelon the first signs were on the lower surface where small green spots appeared and became gray green and round or irregularly shaped with age. At high temperature and humidity, the spots extended rapidly to form large, irregular sunken, grayish-green patches, sometimes blighting the entire cotyledon. On Hubbard squash, reddish-brown to bright orange pustules appeared on the lower surface as soon as the cotyledons emerged. The pustules were usually raised and surrounded by a yellow halo. They were as large as 2 millimeters in diameter and varied in number, the entire cotyledon turning yellow and shriveling in some cases.

The canker on the hypocotyl of Table Queen squash started with an indefinite brownish lesion which increased under favorable humidity and girdled the plant. If humidity was high, damping off might follow, while at times the tissue of the girdling lesion dried out and the top of the plant remained turgid. When seeds were soaked overnight in a suspension of pycnospores from a sporulating culture and planted in sterilized soil the symptoms were similar but the percentage of infected plants was lower.

SUSCEPTIBILITY OF SEEDLINGS

Seeds of Hubbard squash, Table Queen squash, Improved Kleckley Sweet watermelon and Hawkesbury watermelon were treated and planted in sterilized soil. When the first leaves were enlarging, the pots were placed in a moist chamber at 25° C., inoculated with a pycnospores suspension, and left for 48 hours. The pots were then removed to a greenhouse at 28° and low relative humidity. The results, given in table 5, show that watermelon was more susceptible than Hubbard squash and Table Queen squash. The last two developed resistance rapidly following emergence while watermelon remained extremely susceptible. Little or no infection of cotyledons occurred in either species of squash and infection of young leaves appeared as very small yellow hypersensitive lesions which became macroscopically invisible as the leaves enlarged. The cotyledons of watermelon showed local spots or a general blight which extended to the hypocotyl and commonly killed the plant. On watermelon leaves circular or irregularly shaped spots appeared which were gray-green at first and changed eventually to dark brown. Sometimes blight of the whole leaf followed.

TABLE 5.—*Results of inoculation of young seedlings of squash and watermelons with pycnospore suspensions of M. melonis*

Species	Variety	Total plants	Healthy plants	Plants with spotted leaves	Plants with blighted leaves
		Number	*Percent*	*Percent*	*Percent*
Squash	Hubbard	22	63.6	36.4	0
	Table Queen	23	91.4	8.6	0
Watermelon	Improved Kleckley Sweet	25	0	60.0	40.0
	Hawkesbury	32	12.5	40.6	46.9

The inoculation of watermelon was repeated with separate suspensions of ascospores and pycnospores from a profusely sporulating *B–1a* culture secured from monoascosporic isolation from an *As* culture. The two types of spores were segregated by removing perithecia and pycnidia carefully from the culture plates. Both types of spores produced equally heavy infection on cotyledons and leaves.

Seeds of Hawkesbury and Improved Kleckley Sweet varieties of watermelon were sown at different dates so that, for a single inoculation experiment, seedlings at 15, 23, and 30 days after emergence were available. They were inoculated with pycnospore suspension and observed as in previous experiments. The results in table 6 show that in Improved Kleckley Sweet there was a rapid increase in resistance of leaves with age. A similar but much slower progression occurred in Hawkesbury.

As already stated, Grossenbacher (*7*) reported the cucumber as immune to the disease. Weber (*24*) later reported a seedling wilt and leaf spot of that species. Diseased cucumber fruits have been observed frequently in market and transit (*25*). The seedlings of a number of cucumber varieties, including Ohio 31, Maine 2, Chicago Pickling, National Pickling, Stays Green, and F_1 hybrids of crosses between several of these varieties were inoculated through wounds at the first node and kept in a moist chamber at 25° C. for 1 week. None of the plants showed signs of disease. In the next experiment, plants of varieties Long Stays Green, Cubit, Parker, Chicago Pickling, and Marketer were inoculated by spraying with a suspension of pycnospores when the first two foliage leaves were enlarging. The seedlings were placed in the moist chamber 3 days before and kept there 3 days after inoculation and were then removed to a greenhouse at 28° C. Gray-greenish spots developed, beginning near the margins of the cotyledons, and progressing inwardly. Yellowish lesions on leaves were usually very small and either disappeared macroscopically or remained as inconspicuous brown spots. It was obvious that cucumber seedlings were highly resistant in the early seedling stage.

TABLE 6.—*Development of disease on watermelon seedlings at different ages*

Variety	Days after emergence	Leaves inoculated	Healthy leaves	Slightly spotted leaves	Severely spotted leaves	Blighted leaves	Disease index
	Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	
Improved Kleckley Sweet	15	54	25.9	50.0	24.1	0	32.7
	23	22	86.3	13.7	0	0	4.9
	30	20	90.0	10.0	0	0	3.3
Hawkesbury	15	31	29.0	45.1	25.8	0	32.3
	23	21	33.3	38.1	28.6	0	31.8
	30	17	58.8	23.5	17.7	0	17.6

Microscopic examination of inoculated cotyledons showed that the germ tubes of pycnospores rarely penetrated unwounded tissue. An experiment was then planned in which the cotyledons of some of the plants were injured by rubbing with a suspension of carborundum before inoculation. These were compared with plants inoculated in the usual way. The results are given in table 7. There was a wide difference between varieties in the amount of infection in unwounded

cotyledons. Except for Chicago Pickling there was a substantial increase in cotyledon infection following abrasion with carborundum.

In previous experiments the hypocotyl of Table Queen squash was very susceptible in seedlings grown on infested soil, but when pycnospores were sprayed on the hypocotyl, cankers were formed only rarely. Certain other methods of inoculation were tried. Seeds were sown in 6-inch pots, and the plants were thinned to 3 per pot. When the primary leaves were well developed, pycnospores and mycelium were applied separately to hypocotyl wounds. In some cases the plants were placed in the greenhouse at 28° C. without further treatment; in other cases the wounds were wrapped with absorbent cotton which was kept moist for 4 days. The results after 1 week are given in table 8. They show that a high percentage of infected plants was secured when the wounds were kept moist for a period of 4 days.

TABLE 7.—*Results of inoculation of cucumber seedlings in which uninjured cotyledons and cotyledons wounded with a suspension of carborundum were compared*

Variety	Plants infected when—		Increase or decrease in wounded plants as compared with non-wounded plants
	Not wounded	Wounded	
	Percent	*Percent*	*Percent*
Long Stays Green	22	40	+18
Cubit	0	40	+40
Parker	45	75	+30
Chicago Pickling	89	80	−9
Marketer	0	40	+40
Stays Green	0	40	+40

TABLE 8.—*Infection of hypocotyls of Table Queen squash inoculated by several methods*

Treatment	Inoculum	Plants with degree of infection indicated				Disease index
		None	Slight	Severe	Killed	
		Percent	*Percent*	*Percent*	*Percent*	
Cotton applied	Mycelium	4.7	16.3	20.8	58.2	77.8
	Pycnospore	0	0	33.3	66.7	89.0
No cotton applied	Mycelium	42.8	4.8	23.8	28.6	46.1
	Pycnospore	51.7	20.7	13.8	13.8	29.9

TEMPERATURE IN RELATION TO DISEASE DEVELOPMENT

Previously recorded observations on the environmental conditions favorable for the development of black rot are meager. In New York, Grossenbacher (*7*) considered "warm, moist" environment essential to development in the greenhouse, and he regarded field environment in that locality to be generally unfavorable. Orton and Meier (*17*) regarded abundant rainfall and relatively high temperatures essential to the development of field epidemics. Walker and Weber (*23*) noted the severest development of the disease on watermelon in Florida following cold, wet periods. In this investigation, the disease was studied on young plants grown at controlled temperatures in the greenhouse.

Twenty-day seedlings of Hawkesbury and Improved Kleckley Sweet watermelon were placed in inoculation chambers for 2 days at 12°, 16°, 20°, 24°, and 28° C., respectively, and then were inoculated with a suspension of pycnospores from a *B–la* culture. In another experiment 16-day Early Knight muskmelon seedlings in pots were inoculated within the following ranges: 12–16°, 16–20°, 20–24°, and 24–28° C., respectively. The results, given in figure 5, show that 24°

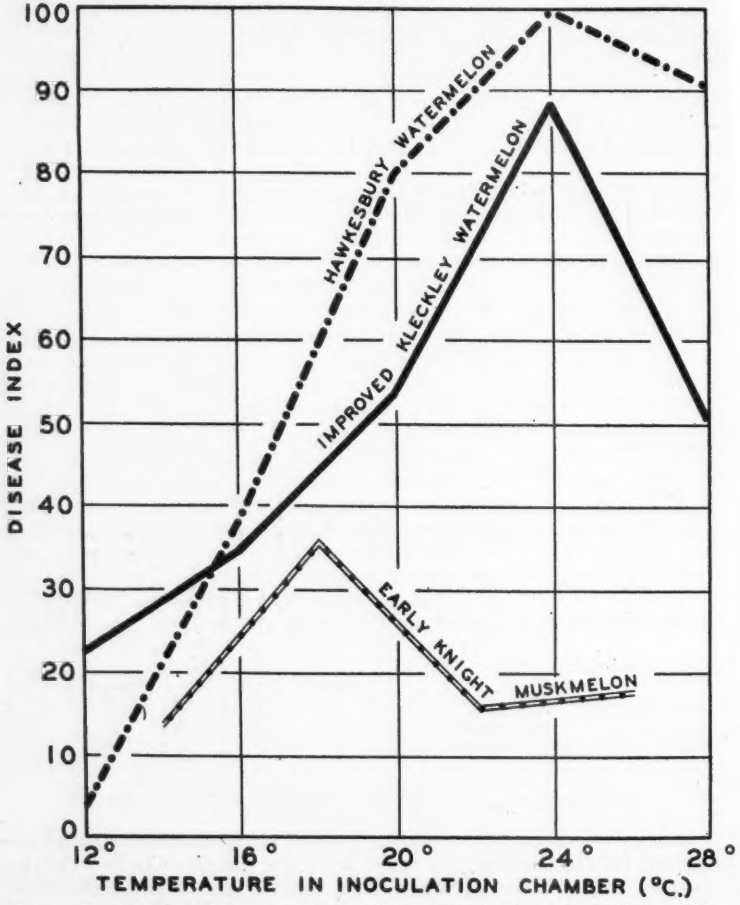

FIGURE 5.—Relation of temperature to disease development in 20-day seedlings inoculated and maintained in a moist chamber for 48 hours.

was the most favorable temperature for infection of watermelon and the disease index decreased steadily with temperature to 12°. In the case of this host the optimum temperature for infection corresponded to that for spore germination. In the case of muskmelon, the disease index was generally low because there was no blighting of

the young shoots, although the leaves were badly spotted. The optimum temperature for infection was some 6 degrees lower than in watermelon. Several trials with the same variety and with Golden Delicious and Wisconsin Pride varieties yielded similar results. A possible explanation for this difference between hosts is that the susceptibiity of watermelon seedlings was constant at all temperatures and consequently the effect of temperature on spore germination and growth was most important, while in muskmelons resistance may have increased with temperature so that the relation of temperature to infection was the result of the effect of temperature on host and on pathogen as well as on their interaction.

In another experiment, 27-day seedlings of Hawkesbury and Improved Kleckley Sweet watermelon were placed for two days in a moist chamber at 24° C., then inoculated and allowed to stay in the moist chamber overnight. They were divided between greenhouses controlled at 16°, 20°, 24°, and 28° C. The disease was very slight at 16° but at the other three temperatures the disease indices were very close (60 to 65). It would appear, therefore, that while the most favorable temperature at the time of inoculation of watermelon is 24°, the disease progresses subsequently about equally well on young plants at temperatures from 20° to 28°.

MODE OF PENETRATION

Grossenbacher (*7*) demonstrated that penetration was possible through the uninjured surface of the muskmelon stem, yet he did not mention the mode of penetration. Klebahn (*10*), in his study of *Ascochyta lycopersici*, claimed that the penetration was direct. Hare and Walker (*8*) also observed direct penetration in *Mycosphaerella pinodes*.

In the present observation, pycnospores were sprayed on cotyledons of watermelon and cucumber seedlings. After 24 hours in a moist chamber at 25° C., the cotyledons were detached and stained with 1-percent crystal violet for 1 minute and immediately washed with running water to remove the superfluous stain. The upper and lower epidermis was then carefully removed with a razor blade and observed directly under the microscope. Since the germinating spores and parts of the germ tubes on the surface of the host took the stain readily while the walls of the epidermal cells of the host did not, the differentiation between parasite and host was very distinct. Stages of penetration are illustrated in figure 6.

In the case of watermelon cotyledons direct penetration took place on both surfaces; no stomatal penetration was found. The relatively thick germ tubes sometimes became slender before penetration. Incidentally, one germ tube sent out two or more branching infection threads to penetrate at several points.

In the case of cucumber cotyledons, most germ tubes of pycnospores grew over the upper surface without a single penetration. On the lower surface, occasional penetration into the intercellular spaces was observed. The base of the abraded trichome on the upper surface was probably a favorable point for infection, but none of the germ tubes of pycnospores was able to penetrate the intact base of the trichome. When the upper surface of the cucumber cotyledon was

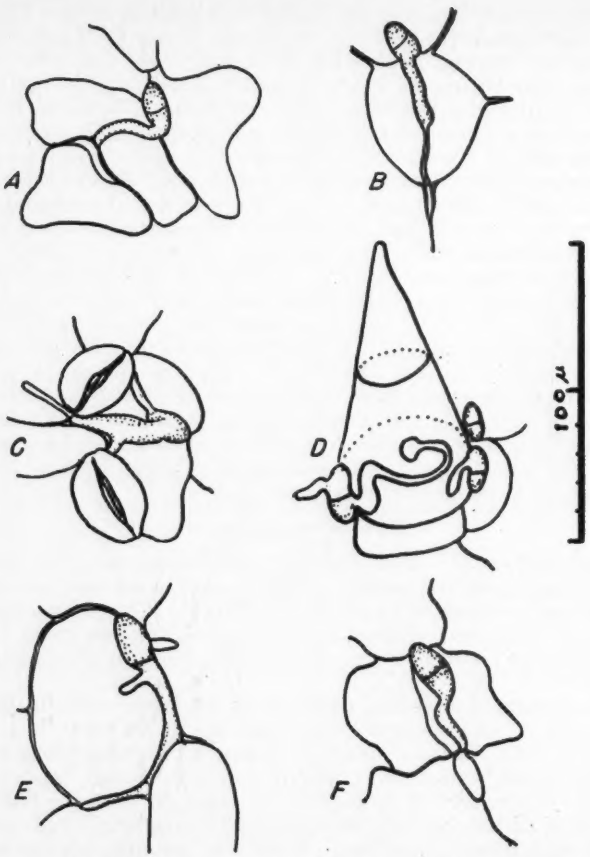

FIGURE 6.—Penetration by hyphae from pycnospores: *A*, *B*, direct penetration between epidermal cells of upper surface of watermelon cotyledon; *C*, penetration of lower epidermis of watermelon cotyledon; *D*, *E*, penetration through base of wounded trichome on upper epidermis of cucumber cotyledon; *F*, penetration between epidermal cells of lower surface of cucumber cotyledon.

abraded with carborundum, the percentage of infection increased, due probably to the increased number of injured trichomes.

Direct penetration of the hypocotyl and the stem of seedlings of Table Queen squash, watermelon, and muskmelon by mycelium was also observed. Penetration did not take place, however, unless the mycelium was placed in close contact with the surface of the hypocotyl or stem and supplied with sufficient moisture by wrapping in moistened cotton for several days. In the field it was observed that the long contact of the procumbent vines of watermelon, muskmelon, and cucumber with the moistened infested soil usually induced development of vine cankers. In nature, most vine cankers began development on the lower side of the stem.

TABLE 9.—*athogenicity of strains of M. melonis when inoculated on hyocotyls of Table Queen squash*

Strain	Plants inoculated	Plants in disease class indicated				Disease index
		Healthy	Slight	Severe	Killed	
	Number	*Percent*	*Percent*	*Percent*	*Percent*	
As	22	0	13.6	63.6	22.8	69.6
A [1]	25	4.0	52.0	36.0	8.0	54.8
A–1 [1]	17	58.8	41.2	0	0	13.6
A–2 [2]	18	0	11.0	22.4	66.6	85.0
A–2s [2]	23	52.2	30.4	8.7	8.7	24.6
B–a [1]	18	0	72.0	22.4	5.6	44.4
B–b [1]	24	0	29.2	45.8	25.0	65.2
B–1a [1]	55	0	5.5	27.5	67.0	87.0
B–1b [1]	24	0	4.1	33.3	62.6	86.5
B–3 [2]	24	100.0	0	0	0	0
Uninoculated	32	100.0	0	0	0	0

[1] Spontaneous mutant.
[2] Mutant which appeared after irradiation.

VARIATION IN PATHOGENICITY

In a previous paper (*4*) it was shown that the wild type (*As*) of the causal organism gave rise spontaneously to mutants and that the rate and type of mutation was modified by irradiation. Some of the mutants were sterile and in others the degree of sporulation varied. In order to carry out an assay for pathogenicity a method was needed in which mycelium and spores would serve equally well as inoculum. Wound inoculation of hypocotyls of young Table Queen squash plants was used for the assay. After several preliminary trials, a standard procedure was selected in which the inoculation period was 72 hours at 28° C., and subsequent incubation in the greenhouse was at 28° to 32°. While the experiment was not set up in such a way that the significance of differences in indices could be calculated, it is obvious that a wide range in pathogenicity prevailed (table 9). Three mutants (*B–1a*, *B–1b*, and *A–2*), one of which was secured after irradiation, were more pathogenic than the wild type, although more data would be necessary to determine whether they were significantly so. *A*, the common mycelial mutant arising from *As*, was slightly less pathogenic than the wild type. One spontaneous mutant (*A–1*) was very low in pathogenicity, while one mutant (*B–3*) isolated after irradiation was nonpathogenic under the conditions of the experiment. *A–2* was a sterile albino while *A–2s* was a fertile albino. There was no apparent linkage between pathogenicity and such cultural characters as pigmentation, sporulation, and growth rate.

INOCULATION OF SEEDLINGS OF OTHER CUCURBITS

Plants of several species and varieties of cucurbits not previously tested were inoculated in the seedling stage in the greenhouse. Some plants were inoculated through the wounds at the cotyledonary nodes, and some by spraying in the first-leaf stage; others were inoculated through wounds on the runner when the latter was about 2 feet in length. The tests were started in July and continued into September 1946. No infection occurred from spraying the cotyledons and the foliage leaves with a suspension of pycnospores except in the case of Dickinson pumpkin, where fairly severe lesions were produced on

cotyledons but not on leaves. The development of cankers on older runners was meager but the results were probably influenced more by the low humidity of the greenhouse than by the inherent resistance or susceptibility of the plants. The list of species and varieties and the results from inoculation at the cotyledonary nodes, which were more consistent than those from other methods, are given in table 10. Cankers were most severe on *Cucurbita foetidissima* Kunth and fairly severe on *C. andreana Naud.* and Small Sugar pumpkin. Small cankers were formed on Dickinson pumpkin and *Early Prolific* (Straight Neck) squash. Limited dead lesions formed around the points of inoculation on *C ficifolia* Bouche, *Lagenaria leucantha* Rusby and Gray Zucchini vegetable marrow.

TABLE 10.—*Species and varieties of cucurbits tested for susceptibility to M. melonis in the seedling stage*

Species	Variety	Infection of cotyledons	Infection of leaves	Cankers formed from inoculation	
				At cotyledonary node	On runners
Citrullus vulgaris		[1] −	−	−	−
Cucurbita andreana		−	−	++	+−
C. ficifolia		−	−	+−	+−
C. foetidissima		−	−	+++	+−
C. pepo	Small Sugar	−	−	++	+
	Dickinson	++	−	+	+
	Gray Zucchini	−	−	+−	+−
C. moschata	Early Prolific (Straight Neck)	−	−	+	+
Lagenaria leucantha		−	−	+−	+−

[1] Explanation of symbols: + indicates the degree of infection; − means no infection; +− means the formation of small and limited lesion.

INOCULATIONS IN THE FIELD

In 1946, inoculations were made on plants in the field at Madison, Wis. Hawkesbury, Improved Kleckley Sweet and Early Kansas watermelon varieties, Hubbard squash, and Table Queen squash were sown in June. In July, inoculum from a *B–1a* culture was inserted in wounds on the hypocotyl, at the cotyledonary node, in the lower stem and near the growing point. In early August small lesions were evident on watermelon around the points of inoculation where a reddish brown gummosis occurred. In early September severe cankers had formed on Hawkesbury plants in the region around the cotyledonary node and fruiting bodies were observed on September 20 (fig. 7). When inoculations were made on the stem two or three nodes above the cotyledonary node cankers also developed in the Hawkesbury plants. Deep cracks in the tissue occurred as the lesion progressed but the relative number of fruiting bodies was lower than in the cotyledonary node (fig. 8). The cankers which formed following inoculation near the growing point were smaller and few fruiting bodies were produced in them. The cankers on plants of the other two watermelon varieties were very small. Little signs of disease occurred on Table Queen squash. On Hubbard squash, lesions appeared at points of inoculation as white patches and the stems became swollen. Cankers were not conspicuous until the fruits began to mature and fruiting bodies on the fungus did not appear until late in October.

In 1947, varieties of watermelon, muskmelon and cucumber listed in table 11 were inoculated. Yellowing and gradual defoliation of the older portion of the vines of Hawkesbury became evident as the first fruits began to mature. This variety was definitely more susceptible than Improved Kleckley Sweet, as it was in the field in 1946 and in the seedling inoculations in the greenhouse reported earlier in this paper. On muskmelon, the cankers were more severe than on watermelon and the amber-colored gummosis was more prevalent.

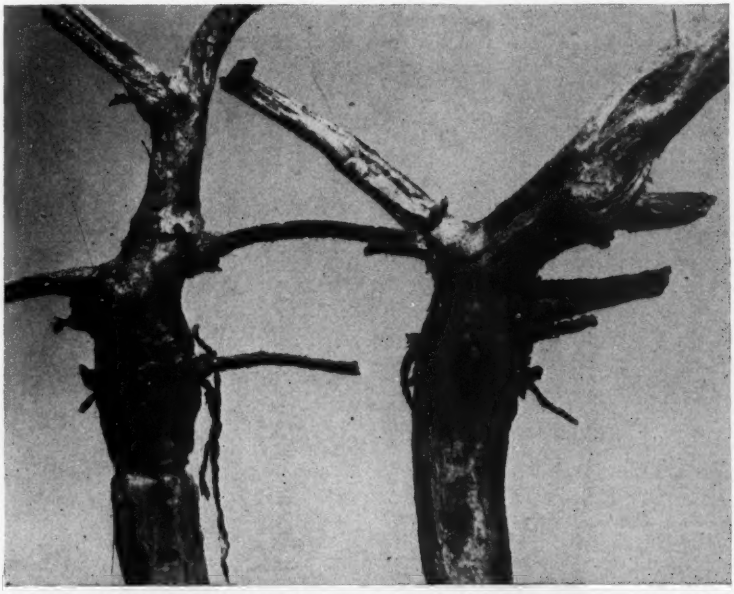

FIGURE 7.—Cankers at the cotyledonary nodes of Hawkesbury watermelon plants near the end of the growing season. The plants had been inoculated in the early seedling stage. The fungus is fruiting abundantly in the lesion.

The yellowing and defoliation of the older portion of the vines occurred earlier than in the watermelon. On cucumber, small lesions appeared at points of inoculation but cankers and fruiting bodies did not appear until late in September (fig. 9). Table 11 shows the extent of infection of vines which had developed by early October. Improved Kleckley Sweet watermelon was more resistant than Hawkesbury watermelon and Wisconsin Pride muskmelon. A wide range of infection occurred in cucumber varieties. Cubit was highly resistant as it was in the seedling inoculations in the greenhouse, while Parker, Stays Green, and National Pickling were very susceptible. Rows of uninoculated plants occurred so that by the end of the season a considerable percentage of diseased plants were present in the uninoculated rows. The records of disease on plants in the uninoculated rows are included in table 11.

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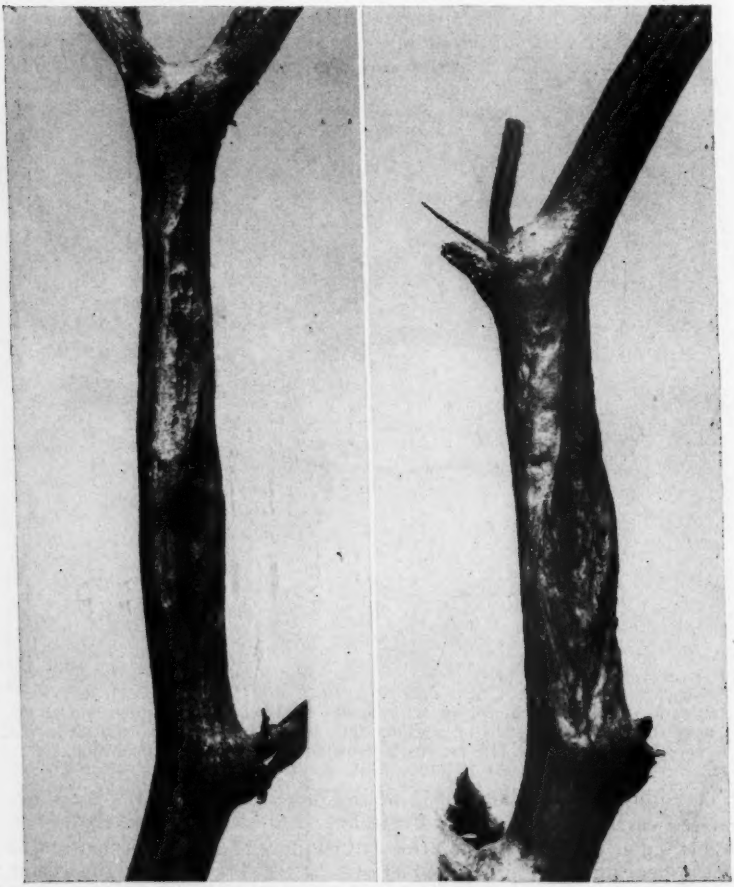

FIGURE 8.–Cankers characterized by deep cracks and relatively few fruiting bodies on the lower stems of Hawkesbury watermelon plants inoculated through wounds.

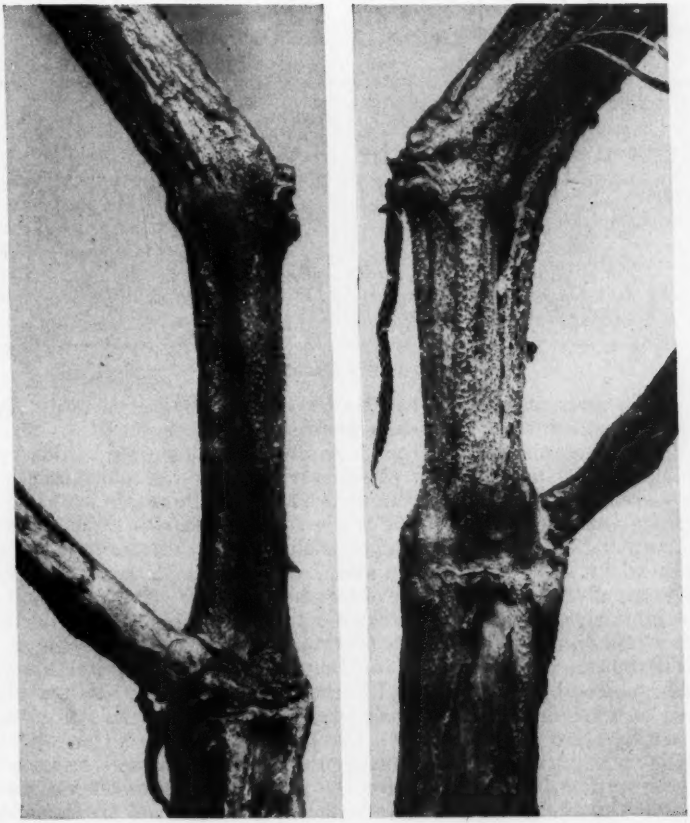

FIGURE 9.—Cankers on the lower stems of cucumber near the end of the growing season. The plants were inoculated through wounds in the early seedling stage. The fungus is fruiting abundantly.

TABLE 11.—*Occurrence of black rot on plants of watermelon, muskmelon, and cucumber varieties, following inoculation in the field at Madison, Wis., in 1947*

Species	Variety	Plants in disease class indicated and the disease index for the lot when—							
		Inoculated				In adjacent, uninoculated			
		Healthy	Slightly cankered	Severely cankered	Disease index	Healthy	Slightly cankered	Severely cankered	Disease index
		Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*	
Watermelon.	Hawkesbury	0	10	90	94	0	100	0	33
	Improved Kleckley Sweet.	0	92	8	38	100	0	0	0
Muskmelon.	Wisconsin Pride.	0	0	0	100	3	97	0	32
	Stays Green	0	38	62	75	0	77	23	49
	Parker	20	10	70	74	54	39	7	25
Cucumber.	National Pickling.	0	32	68	71	18	30	52	63
	Chicago Pickling.	39	4	57	58	72	20	8	15
	Long Stays Green.	50	12	38	48	74	17	9	15
	Cubit	65	26	9	12	96	0	4	4

DISCUSSION

In the present work, it has been pointed out that to insure full vigorous germination of the ascospores and pycnospores of the black-rot fungus, a minute quantity of stimulant was required. Although orange extract and cucurbit tissue extracts have the same function, they are considered to be different in nature. The active material in orange extract is thermostable while that in cucurbit tissue extract is thermolabile. Carbon from sucrose and mineral salts may not be essential for spore germination and the presence of a complete nutrient is not effective as in the case of *Glomerella cingulata* (*12*). Instead, the inhibitive effect of a nutrient solution as observed earlier by others and discussed by Duggar (*6*), was experienced.

The optimum temperature for fungus growth was quite close to that reported by Wiant (*25*). It is interesting to note that it is also close to those of *Mycosphaerella pinodes* and *Ascochyta pisi* as reported by Hare and Walker (*8*).

The optimum temperature for pycnospore germination corresponds to the optimum temperature for the infection of watermelon seedlings. This indicates that the influence on infection in this case was based on the effect of temperature on the pathogen. In the case of muskmelon, however, the optimum temperature for infection was lower, which may be due to a higher resistance of the muskmelon at the optimum temperature for the pathogen.

Reports regarding the development of the disease in the field on watermelon plants vary. Walker and Weber (*23*) reported disease to be most severe in cool and wet weather in Florida, while Orton and Meier (*17*) reported that vines were killed in warm and rainy periods. The present studies show that temperature and humidity are critical factors in infection and that the high air humidity or soil moisture exerts a greater favorable influence on disease development than temperature. Observations in the greenhouse and in the field showed that infected cotyledons became a source of further inoculum for infection

of the hypocotyl and stem, where severe cankers were formed when infected portions were in contact with moist soil. The development of such cankers was rapid under humid conditions in the greenhouse as low as 16° C. On the basis of these findings it is thought that the prevailing wet weather was the main influential factor in observations by Walker and Weber (*23*). In general, the observations of Grossenbacher (*7*) and of Orton and Meier (*17*) coincide with the results of the present work.

Variability in cultural characters was accompanied by variation in pathogenicity. Further studies on this phase of the problem should be made and extended to collections of the fungus from various cucurbits from different localities. It is possible that the strains used in this investigation are more pathogenic on watermelon than some others which might be found to exist. The development of resistant varieties of watermelon and cucumber may be possible. It has not been determined, however, whether plants with resistant vines produce resistant fruits. Nevertheless reduction of the amount of inoculum on vines might be a factor in the reduction of infection of fruits and thus contribute toward reduction of the disease in market and transit.

SUMMARY

The requirements for ascospore and pycnospore germination were studied. Small quantities of orange extract or cucurbit seed-cotyledon extract added to the medium, stimulated germination. The optimum temperature for germination of pycnospores was 24° C. Dextrose solution stimulated germination slightly but not as well as orange extract. The effect was additive and the highest percentage occurred at M/1 concentration. Sucrose had an inhibitive effect on pycnospore germination and the effect was also additive. A nutrient solution containing all essential minerals and assimilative carbon inhibited germination somewhat in the presence of orange extract.

When toxicity tests were carried out in petri dishes, silver nitrate was the most toxic substance tested. It required as low as 0.01 gamma of silver per millimeter to inhibit 95 percent of germination. Mercuric chloride was also highly toxic but far less so than silver nitrate. Copper sulfate, although comparatively low in toxicity in comparison with silver nitrate and mercuric chloride, was still a promising toxicant. It required 4.3 to 19 gamma of copper to inhibit 95 percent of the spores. The LD95 of copper varied with the conditions under which the pycnospores were germinated. Zinc sulfate was inferior to copper sulfate as a toxicant, while the results with aluminum sulfate were inconclusive.

Optimum fungus growth on agar occurred at 20° to 24° C., with the upper limit 36° C., and very slow growth at 12° C. Growth was not influenced by the initial pH value of the medium except at values below pH4. In the fertile strain, *As*, sporulation was most profuse at pH7.

The fungus overwintered at Madison, Wis., as dormant mycelium in infected cucurbit debris.

The pycnospores and mycelium were equally infective on cotyledons and on young leaves of muskmelon and watermelon. Leaves of cucumber, Hubbard squash, and Table Queen squash were very resistant. Infection of the hypocotyl and the stem of all cucurbits tested occurred

only though punctures, through weakened areas, or by extension of the lesions on the cotyledon. Cucumber and Hubbard squash were resistant to formation of cankers on the hypocotyl when young, but both became very susceptible when old. The contrary was true for Table Queen squash. Under moist conditions cankers developed on the stems and hypoctyls of watermelon and muskmelon throughout their growing periods.

Ten strains of the pathogen secured by single-spore isolations, some from untreated and some from irradiated cultures, showed a gradation from very high pathogenicity to none when tested on hypocotyls of Table Queen squash.

Penetration of the host tissue was direct or through intercellular spaces around the basal cell of the abraded trichome.

The optimum temperature for infection of watermelon was 24° C. and for infection of muskmelon was 16° to 20° C. The temperature after the establishment of the infection was less important than the relative humidity.

On severely infected watermelon vines in the field the disease caused yellowing and defoliation of the plant, beginning at the center of the hill. Other symptoms in the field resembled those produced by drought injury. Field experiments revealed that Improved Kleckley Sweet watermelon was decidedly more resistant than Hawkesbury. Wisconsin Pride muskmelon was equal to Hawkesbury watermelon in susceptibility. Cubit variety of cucumber was much more resistant than Stays Green, Chicago Pickling, Parker, Marketer, Long Stays Green, and National Pickling.

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WISCONSIN PEA STUNT, A NEWLY DESCRIBED DISEASE[1]

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INTRODUCTION

Although the severity of pea virus diseases in Wisconsin varies from year to year, they are sometimes the cause of serious reductions in yield. These losses are almost always more severe during the last 2 weeks of the canning season and may be associated with abundant and active aphid populations.

To determine what pea viruses were prevalent in southern Wisconsin in 1946, several fields were examined and isolations attempted from peas (*Pisum sativum* L.) affected with virus disease. One of the isolations made was from a severely stunted plant found in the pea nursery at Madison. A considerable number of similarly diseased pea plants have been observed in Wisconsin pea fields during the last three seasons. In tests made during the summer of 1948, the stunt virus was isolated from 11 different pea fields widely scattered over the State. This would indicate that the virus to be considered here is common in Wisconsin and may be considered a component of the virus complex that is sometimes troublesome.

Detailed study of the pea stunt virus isolated in 1946 indicated that it was different from any previously reported virus of peas. It is designated the Wisconsin pea stunt virus. The purpose of this paper is to describe and identify the virus and to submit experimental data regarding symptoms, temperature relations, host range, varietal reactions, physical properties, and transmission.

METHODS AND MATERIALS

Throughout most of the greenhouse studies to be described, the plants were grown in compost soil in 6-inch earthenware pots at temperatures of from 20° to 24° C. The Perfected Wales variety of pea was used as a culture plant, and for most of the experiments, because it was very susceptible to the virus. Several of the less common legume seeds used were obtained from Roland McKee of the United States Department of Agriculture.

The best inoculation was obtained when the leaves were rubbed with a pad made by folding cheesecloth around macerated diseased tissue.

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[2] The writers are indebted to Eugene H. Herrling for making the photographs used in this manuscript. The senior author expresses appreciation to W. W. Hare for helpful advice and material aid.

Carborundum powder, sprinkled lightly on the leaves, greatly aided infection. The second and third leaves of pea were inoculated when they became fully expanded. Other hosts were inoculated similarly when they were young.

Physical properties were determined in a manner similar to that described by Johnson and Grant [3] except that aging tests were conducted at a temperature of about 22° C. (72° F.) instead of 80–90° F.

The pea aphids (*Illinoia pisi* (Kalt.)) used for insect-transmission studies were always tested to make sure they were free from virus, and were maintained either on broadbeans (*Vicia faba* L.) or on peas in an aphid-proof wire-mesh cage. Such cages were used for detention of insects during the tests conducted. Healthy insects were allowed to feed on diseased plants for 2 days and then were transferred to young healthy plants, usually for a period of 2 days. Six to 14 aphids were placed on each plant. Insects were confined to the plants by means of glass lamp chimneys. In most cases aphids were transferred by cutting off the leaf on which they were feeding and removing it to the desired location. At the end of the allotted feeding time on healthy plants, the insects were killed by spraying with an aphicide.

Suitable controls were provided for all experiments conducted.

EXPERIMENTAL RESULTS

SYMPTOMS

The most striking symptoms of the Wisconsin pea stunt disease in the field were severe stunting of the plant (fig. 1) and malformation of the terminal foliage into a tight rosette of the leaves, stipules, and blossoms (fig. 2). Internode lengths were so greatly reduced that the stunted vines were often only one-fourth to one-half the height of healthy plants. The leaves and stipules of the rosette were reduced in size and failed to open properly. They were often wrinkled somewhat and showed definite vein clearing. Stipules and leaves just below the rosette did not open fully and attain normal size. Instead, they were wrinkled and folded upward. These leaves also showed a conspicuous vein clearing. Middle and lower leaves were slightly chlorotic.

Axillary bud growth was very common and sometimes quite profuse. Very few pods were found on infected plants and those examined were poorly filled. In cases where pods were formed, the plant was thought to be infected at a later stage as compared with other diseased plants. Leaves of the lower and middle stem sometimes showed browning of the veins. No mottling was observed.

The first noticeable symptom of pea stunt on inoculated plants in the greenhouse was a yellowing and wilting of the inoculated leaves. This symptom occurred in 12 to 16 days after inoculation. Stunting of the plant and inward curling of apical leaves were evident about the same time. A faint tannish-green discoloration of the stem was sometimes detected a little later. Apical leaves and stipules showed vein clearing and inward, upward curling of the upper leaves often was to be seen. The typical malformation of the apical leaves into a

[3] JOHNSON, J. and GRANT, T. J. THE PROPERTIES OF PLANT VIRUSES FROM DIFFERENT HOST SPECIES. Phytopath. 22: 741–757. 1932.

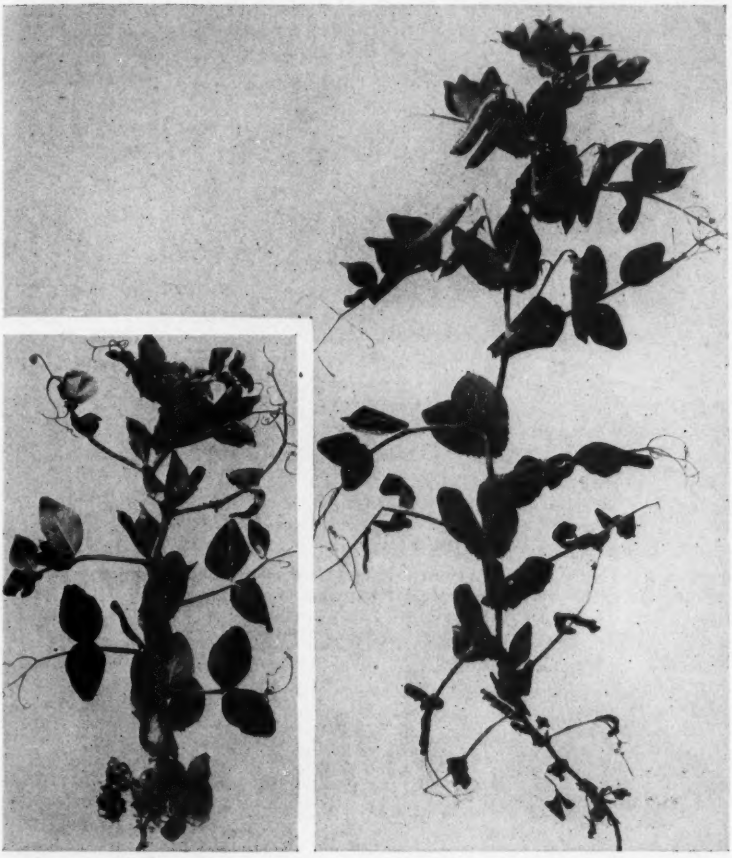

FIGURE 1.—Symptoms of the Wisconsin pea stunt virus in the field. The diseased plant (left) shows severe stunt, shortened internodes, and severely rosetted apical foliage compared to healthy plant (right).

tight rosette was noted from 16 to 20 days after inoculation. In many cases the height of the plants infected with this virus was only 6 to 10 inches, when healthy plants were sometimes twice as high. This reduction of plant size was due largely to greatly shortened internodes. Axillary bud growth was profuse in most instances. Diseased plants often died 20 to 26 days after inoculation from wilting which had progressed upward. The axillary bud growth occasionally survived for some time after the main stem had wilted and died.

The Wisconsin pea stunt virus on red clover (*Trifolium pratense* L.) caused a slight reduction of leaf size and a distinct roughening and wrinkling of leaves (fig. 3, *B*). A very slight mottle also was seen. On alsike clover (*T. hybridum* L.) this virus produced necrotic chlorosis of the veins and slight inward curling of the leaflet edges (fig. 3, *A*).

FIGURE 2.—A view from above of the apical foliage rosette seen in figure 1. Note that the veins are clear but that there is no mottle.

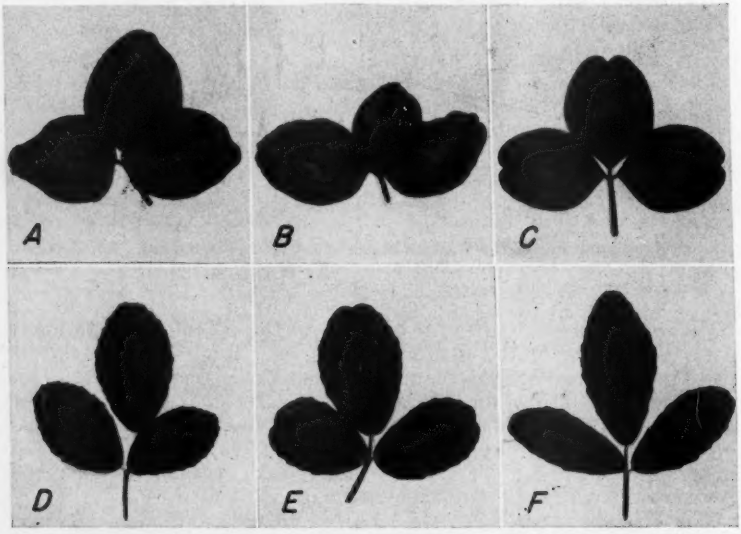

FIGURE 3.—Symptoms of the Wisconsin pea stunt virus on clover leaves. *A*, Diseased alsike clover; *B*, diseased red clover; *C*, healthy red clover; *D*, diseased white sweet clover; *E*, diseased yellow sweet clover; *F*, healthy yellow sweet clover.

White sweet clover (*Melilotus alba* Desr.) leaves showed striking mosaic symptoms when infected with the stunt virus (fig. 3, *D*). This mosaic resulted from the development of conspicuous chlorotic spots along the veins and occasionally between them. Most of these narrow, yellowed places were oblong in shape, had definite, irregular margins, and sometimes surrounded a small area of normal green tissue. Vein clearing was the characteristic symptom produced when yellow sweet clover (*M. officinalis* (L.) Lam.) was infected with this virus (fig. 3, *E*).

RELATION OF TEMPERATURE TO DISEASE DEVELOPMENT

Low temperature had a temporary masking effect on pea stunt symptoms, for when pea plants inoculated with the causal virus were grown at 16° C. the first symptom did not occur until 33 days after inoculation. This was more than twice the time necessary to bring forth symptoms at 20°, 24°, and 28°.

Infected plants growing at 16° C. first showed moderate vein clearing and about half of them were malformed slightly at the growing tip. Very slight stunting was seen in some of the diseased plants. As the disease progressed, severe vein clearing took place and the plants showing apical malformation (some diseased plants showed vein clearing only) developed a more apparent distortion. Moderate axillary bud growth was noted. Small tannish-gray discolorations of the stem and slight browning at the nodes were also present. Most diseased plants were dead 66 days after inoculation.

At 20° C. typical pea stunt symptoms were seen 15 days after inoculation. Diseased plants showed more apparent stunting and apical malformation than at 16° C., and fewer and less pronounced necrotic stem discolorations. Moderate node browning, axillary bud growth, and vein clearing were present. Death of most infected plants had occurred 36 days after inoculation.

When the temperature study was made at 24° C. there was an incubation period of 12 days. A rather tight rosette of apical leaves developed and stunting of the plant was very pronounced. Moderate vein clearing and axillary bud growth were noted as well as slight browning at the nodes. All diseased plants had died 27 days after inoculation.

Symptoms of pea stunt were apparent 11 days after inoculation when plants were grown at 28° C. Very severe plant stunting and malformation of apical foliage into a tight rosette were characteristic of the disease at this temperature. The plants wilted about 20 days after inoculation and died shortly thereafter.

The effect of temperature on the apical foliage symptoms is shown in figure 4.

HOST RANGE

Twenty-nine members of the Leguminosae, Solanaceae, and Cucurbitaceae, were tested for susceptibility to the Wisconsin pea stunt virus. The plants tested and the results obtained are given in table 1. Inoculations from the various test plants back to healthy Perfected Wales peas were made to check the presence or absence of the virus. When the virus was recovered, even though no symptoms were observed on the host it was indicated as "latent." If symptoms were absent or questionable and the virus was not recovered, the host was considered nonsusceptible.

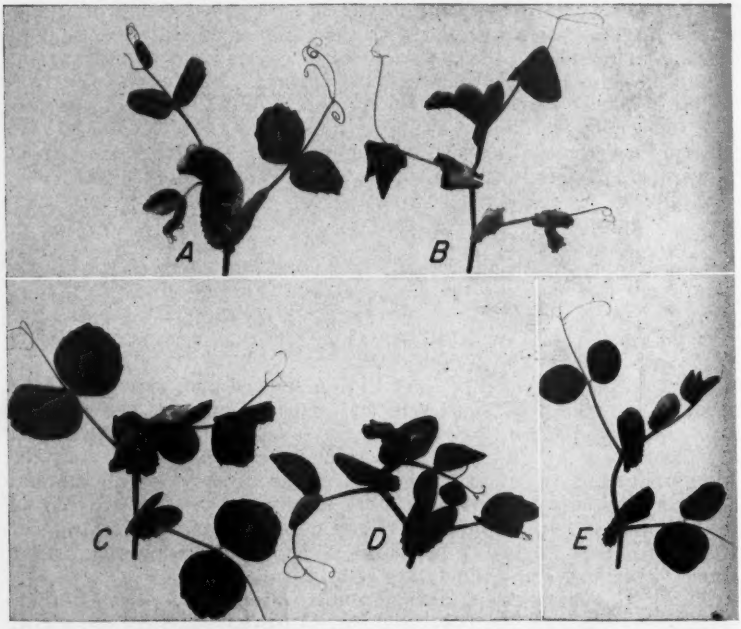

FIGURE 4.—The effect of temperature on the apical foliage symptoms produced by the Wisconsin pea stunt virus at *A*, 16° C.; *B*, 20° C.; *C*, 24° C.; *D*, 28° C. *E*, Healthy plant at 20° C. Vein clearing is the most conspicuous symptom at 16° C., but as temperature rises the severity of the apical malformation increases.

TABLE 1.—*Host range and symptoms of Wisconsin pea stunt virus*

Host	Plants inoculated	Plants diseased	Typical symptom
	Number	*Number*	
Lathyrus hirsutus L.	22	4	Faint mottle.
L. odoratus L.	11	9	Apical malformation.
Lupinus angustifolius L.	4	(1)	
Medicago orbicularis All.	4	0	
M. sativa L.	17	0	
Melilotus alba Desr.	14	9	Definite mottle.
M. officinalis (L.) Lam.	14	9	Vein clearing.
Phaseolus vulgaris L.	27	0	
Pisum sativum L.	31	23	Plant stunt.
P. sativum var. *arvense* Poir.	8	1	Apical malformation.
Soja max (L.) Piper	8	0	
Trifolium hybridum L.	16	11	Chlorotic vein necrosis.
T. incarnatum L.	20	15	Vein clearing.
T. pratense L.	40	29	Leaf wrinkle, vein clearing.
T. repens L. (Ladino White Clover)	10	0	
T. repens L. (White Clover)	9	0	
Trigonella foenum-graecum L.	10	9	Vein clearing.
Vicia atropurpurea Desf.	18	(1)	
V. faba L. var. *major*	13	0	
V. faba L. var. *minor*	8	0	
V. grandiflora Scop.	10	2	Faint mottle.
V. monantha (L.) Desf.	16	(1)	
V. sativa L.	6	2	Brown stem necrosis.
V. villosa Roth	11	0	
Vigna sinensis Endl.	10	0	
Cucumis sativus L.	12	0	
Datura stramonium L.	29	0	
Lycopersicon esculentum Mill.	5	0	
Nicotiana tabacum L.	9	0	
Solanum tuberosum L.	4	0	

[1] Latent=no symptoms, but virus recovered.

The virus was limited in host range to the leguminous plants tested. It was not infectious to the following members of the Solanaceae: Jimsonweed (*Datura stramonium* L.); tomato (*Lycopersicon esculentum* Mill.); tobacco (*Nicotiana tabacum* L.); and potato (*Solanum tuberosum* L.). Cucumber (*Cucumis sativus* L.) was also nonsusceptible.

Legumes that showed no susceptibility to the stunt virus were: Button medic (*Medicago orbicularis* All.); alfalfa (*M. sativa* L.); bean (*Phaseolus vulgaris* L.); soybean (*Soja max* (L.) Piper); white clover (*Trifolium repens* L.); broadbean (*Vicia faba* L.); hairy vetch (*Vicia villosa* Roth.); and cowpea (*Vigna sinensis* Endl.). One plant of ladino white clover (*T. repens* L.) developed mottle symptoms suggesting infection, but no virus was recovered.

Only three of the 15 hosts susceptible to the virus failed to produce symptoms. They were blue lupine (*Lupinus angustifolius* L.), purple vetch (*Vicia atropurpurea* Desf.), and monantha vetch (*V. monantha* (L.) Desf.). The other susceptible legume hosts included rough peavine (*Lathyrus hirsutus* L.), sweet pea (*L. odoratus* L.), white sweet clover (*Melilotus alba* Desr.), yellow sweet clover (*M. officinalis* (L.) Lam.), field pea (*Pisum sativum* var. *arvense* Poir.), alsike clover (*Trifolium hybridum* L.), crimson clover (*incarnatum* L.), red clover (*T. pratense* L.) fenugreek (*Trigonella foenum-graecum* L.), showy vetch (*Vicia grandiflora* Scop.), and spring vetch (*V. sativa* L.).

VARIETAL SUSCEPTIBILITY IN PEA AND BEAN

The varietal reactions of 18 pea varieties to Wisconsin pea stunt virus were studied in the greenhouse. Varieties were not considered susceptible unless the virus was recovered. The results of two separate trials are combined in table 2. All 18 varieties were susceptible to this virus and symptoms on all varieties were quite similar.

None of the 11 bean varieties listed in table 2 were infected by this virus. Although one plant of the black-seeded Stringless Blue Lake variety developed a mottle, an attempt to recover the virus from that plant was unsuccessful. Otherwise, disease symptoms were not observed, nor could the pea stunt virus be recovered from any of the inoculated bean plants.

TABLE 2.—*Varietal reactions of pea and bean to the Wisconsin pea stunt virus*

Pea variety	Plants inoculated	Plants diseased	Bean variety	Plants inoculated	Plants diseased
	Number	*Number*		*Number*	*Number*
Alaska	29	2	Bountiful	8	0
Alderman	25	1	Dwarf Horticultural	3	0
Bonneville	19	6	Great Northern U. of I. No. 1	3	0
Canner King	19	9	Idaho Refugee	3	0
Delwiche Commando	18	6	Michelite	5	0
Glacier	21	4	Pinto	9	0
Horal	20	3	Red Kidney	17	0
Improved Penin	14	9	Sensation Refugee 1066	9	0
Loyalty	12	5	Stringless Blue Lake (Black)	3	0
Merit	14	6	Stringless Blue Lake (White)	3	0
Perfected Wales	41	12	Stringless Green Refugee	6	0
Pride	14	6			
Profusion	17	7			
Resistant Famous	22	12			
Thomas Laxton	25	2			
Wasatch	15	5			
Wisconsin Early Sweet	27	6			
Wisconsin Perfection	15	8			

PHYSICAL PROPERTIES OF THE VIRUS

During the course of this investigation a series of experiments was conducted to determine physical properties of the virus in question. The following properties were studied: tolerance to dilution, resistance to aging *in vitro*, thermal inactivation, and longevity in frozen plant tissue. Table 3 gives the results obtained in the study of these properties.

TABLE 3.—*Physical properties of Wisconsin pea stunt virus*

Property test	Trial 1		Trial 2		Trial 3		Property test	Trial 1		Trial 2	
	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased		Plants inoculated	Plants diseased	Plants inoculated	Plants diseased
	Number	*Number*	*Number*	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
Dilution:							Heat: (° C.):				
0	21	16	11	11	9	3	0 (control)	21	11	9	3
1:10	11	6	22	22	4	3	54	19	8	5	1
1:100	11	3	21	16	8	6	56	22	4	7	2
1:1,000	12	1	20	5	9	1	58	21	0	9	0
1:10,000	10	0	20	0	8	1	60	20	0	11	0
1:100,000	12	0	19	0	10	0	62	22	0	7	0
Aging *in vitro*:							64	20	0	10	0
0	10	7	11	10	10	6	Freezing *in vivo*:				
12 hours	4	2	10	5	------	------	0	11	10	------	------
24 hours	8	1	13	2	10	2	5 days	11	0	------	------
2 days	------	------	10	0	11	0	10 days	10	3	------	------
4 days	------	------	------	------	------	------					

When infective juice containing the Wisconsin pea stunt virus was diluted an end point of 1:10,000 was obtained in the first two trials. However, in a third trial, one plant out of eight inoculated with a dilution of 1:10,000 developed typical stunt symptoms, indicating that this virus is not always inactivated until a dilution of 1:100,000 is reached.

All three tests with the stunt virus indicated that this virus was capable of withstanding aging *in vitro* for 24 hours. Five out of thirty-one plants were diseased when inoculated with infective juice which had stood for that time. When longer aging periods were tried no infection was obtained.

In both thermal inactivation tests conducted with this virus it was rendered inactive at 58° C. and the indications are that it is inactivated between 56° C. and 58° C.

The virus was still active in plants frozen for a period of 10 days.

TRANSMISSION

Insect transmission tests were conducted with the pea aphid. In one trial, where infective aphids were placed on five healthy plants, four of these plants became diseased showing typical Wisconsin pea stunt symptoms. The causal virus was recovered. Identical results were obtained when a second trial was made. Thus, the pea aphid was found to be a vector of the Wisconsin pea stunt virus.

Because diseased plants in the field rarely develop seed, only a small seed transmission test was possible. Of 37 plants, which grew from

seeds harvested from stunted pea plants, all failed to develop virus symptoms under optimum greenhouse conditions.

DISCUSSION

Information presented here on the Wisconsin pea stunt virus is believed sufficient to substantiate its identification as a new pea virus. In the following paragraphs, a comparison of this virus is made with some other pea viruses that resemble it most closely.

The only virus previously reported as a pea stunt virus was described by Zaumeyer [4] in 1940. He called the disease "pea stunt mosaic" and the causal virus "pea mosaic virus 5." When Wisconsin pea stunt virus is compared with it, one finds that differences exist in symptoms, thermal death point, pea varietal reaction, and host range. The pea-mosaic virus 5 produced a mottle on pea leaves, but this symptom was never observed on plants diseased with Wisconsin pea stunt. The thermal death point of the former was 62° C., but the pea stunt virus described here was inactivated at 58° C. Zaumeyer's virus did not infect Horal, Wisconsin Early Sweet, or Perfection Peas, but they were all susceptible to the Wisconsin stunt virus. Bean and alfalfa were hosts of pea mosaic virus 5 but it did not infect red clover. An opposite reaction on these hosts was found with the Wisconsin stunt virus.

Of the other known pea viruses, those which most resemble the Wisconsin pea-stunt virus are grouped by Weiss [5] in the pea common mosaic-virus group. This virus group, however, has a dilution end point of 1:1,500 compared to 1:100,000, and is thermally inactivated between 60° and 64° C. This is in contrast to the Wisconsin stunt virus, which is inactivated at 58° C. Foliage mottle is produced on peas by members of the common mosaic-virus group but not by the pea stunt virus. Premature death does not ordinarily occur in plants artificially infected with the common mosaic virus, but those inoculated with the Wisconsin stunt virus always died before pods were formed. Viruses in the pea common mosaic-virus group were infectious to broadbean, but this was not true of the Wisconsin pea stunt virus.

The Wisconsin pea stunt virus differs from Weiss' [5] pea enation mosaic-virus group in the following ways: (1) no enations on leaves or pods are produced by the pea-stunt virus; (2) broadbean and soybean are susceptible, red clover nonsusceptible to the enation viruses, but the opposite is true of the Wisconsin pea stunt virus; (3) the pea stunt virus had a higher tolerance to dilution and a shorter period of activity *in vitro*.

The pea wilt virus described by Johnson [6] in 1942 could possibly be mistaken for the Wisconsin pea stunt virus. However, it is different from the stunt virus in several aspects, being infectious to white clover, bean, broadbean, and cowpea. This host reaction is the opposite of

[4] ZAUMEYER, W. J. THREE PREVIOSLY UNDESCRIBED VIRUSES OF PEA. Jour. Agr. Res. 60: 433–452, illus. 1940.

[5] WEISS, FREEMAN. VIRUSES DESCRIBED PRIMARILY ON LEGUMINOUS VEGETABLE AND FORAGE CROPS. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. Sup. 154: 51–53. 1945. (Mimeographed.)

[6] JOHNSON, FOLKE. THE COMPLEX NATURE OF WHITE CLOVER MOSAIC. Phytopath. 32: 103–116. 1942.

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the host reaction of Wisconsin pea stunt virus. No symptoms are produced in alsike clover by the pea wilt virus but the pea stunt virus causes necrotic veinal necrosis on leaves of that host. The former virus is not transmitted by the pea aphid, but that insect is a ready vector of the latter. The pea wilt virus is infectious after aging *in vitro* for 31 days and the Wisconsin pea stunt virus after aging 1 day.

SUMMARY

An apparently new pea virus, the Wisconsin pea stunt virus, is described. It represents the causal agent of a prevalent virus disease of peas found in southern Wisconsin during recent years.

Symptom descriptions on important hosts are given. On peas, main symptoms are severe plant stunt, rosette of the apical foliage, and premature death.

Low temperature had a masking effect on Wisconsin pea stunt symptoms. Severity of the apical deformation caused by the pea stunt virus increased with rise in temperature.

The virus was limited in host range to the Leguminosae.

All 18 pea varieties tested were infected but none of the 11 bean varieties studied were found to be susceptible.

The Wisconsin pea stunt virus had a dilution end point of 1:100,000, a resistance to aging *in vitro* of 24 to 48 hours, and was thermally inactivated at 56° to 58° C. It was still virulent after 10 days' freezing.

The pea aphid (*Illinoia pisi* (Kalt.)) was found to be a vector of the virus.

PROCEDURES THAT REDUCE DARKENING OF COOKED POTATOES[1] [2]

By Ferne Bowman, *research assistant* and Flora Hanning, *professor of Home Economics, Wisconsin Agricultural Experiment Station*

INTRODUCTION

Blackening of potatoes after cooking is unsightly and markedly affects their market value and consumer acceptance. Ultimately, any appraisal of a food must be based on the characteristics consumers prefer (*5*).[3] Several studies of American consumers' preferences tabulate the cooking qualities in potatoes considered most desirable. In 1,032 home interviews in the cities of Cleveland and Rochester (*8*), mealiness was the quality mentioned by more than three-fourths of the homemakers; whiteness was next in preference. The most common complaint of 880 retailers was the tendency of some potatoes to cook black. In a consumer study of 1,653 individuals in New Hampshire, Rinear (*16*) found that 71 percent stressed mealiness and 14.1 percent stressed white appearance as the cooking quality most desirable for potatoes. Spangler (*19*) reported that of 1,165 Chicago retailers, 25 percent listed a tendency to cook black as either the first or the second objectionable cooking defect of potatoes. In a survey of potato preferences among 1,203 household consumers by the Bureau of Agricultural Economics (*27*), 16 percent mentioned "cooked black" as a frequent defect.

Blackening after cooking is a common fault in potatoes grown in certain northern areas. This discoloration does not appear before and during cooking but develops gradually upon exposure of the potatoes to air after cooking, thus reducing their desirability for home and institutional use. The discoloration most often appears first at the stem-end as a gray or blue-gray color. The area may extend over much of the surface and through the vascular regions and even into the interior of the tuber or it may be quite localized. The color varies from a pale blue-gray to a very dark or even black discoloration. If discoloration takes place, it is more intense if the potatoes are steamed or if they are boiled before peeling.

[1] Appreciation is expressed for the cooperation of Dr. H. M. Darling, of the Department of Plant Pathology, and Dr. G. H. Rieman of the Genetics Department, who supplied the potatoes used in this study, and to Dr. J. K. Arnold of the Mathematics Department, who made the statistical analysis.

[2] Published with the approval of the Associate Director of the Wisconsin Agricultural Experiment Station.

[3] Italic numbers in parentheses refer to Literature Cited, p. 635.

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A number of investigators have successfully reduced the extent and intensity of discoloration in boiled potatoes by adding certain acids to the cooking water. Tinkler (*22*) found that if potatoes which were susceptible to blackening were steamed with ammonium carbonate vapor, darkening was intensified but that potatoes cooked in water containing a little acetic acid were usually better than those cooked in tap water. Gleason and Marlatt (*6*) used 1 part vinegar to 30 parts of water, which lessened darkening but prevented the potatoes from cooking tender. Hansen (*7*) cooked potatoes in acidified waters, and although the color improved, the tubers were not tender. Nutting and Pfund (*14*) boiled potatoes in waters to which either citric acid, lemon juice, or vinegar was added. If the acid was sufficient to give the water a pH value of 4.1 or less before cooking, and not more than 4.9 after cooking, the tendency to blacken decreased or was even prevented. Potatoes cooked in waters with such low pH levels were not palatable because tough layers formed on the outside. Darkening was unchanged or slightly intensified by the use of cooking waters pH 8.4 to 10.

Smith, Nash, and Dittman (*20*) found that the correlation between hydrogen-ion concentration in tuber tissues and the nature and extent of darkening after cooking was high. If potatoes were cooked in dilute solutions of hydrochloric acid and sodium dihydrogen phosphate, pH 4.5, they did not turn dark. Similar potatoes boiled in a disodium phosphate solution, pH 9.6, discolored extensively. Smith and Kelly (*21*) blanched potatoes prior to dehydration in various acid solutions to reduce greying. These workers tested acetic, citric, lactic, orthophosphoric, dihydrogen phosphate, and sulphurous acids and recommended orthophosphoric acid because it was easy to handle, harmless, and cheap.

Thus, there was ample evidence that adding acids to the water when boiling potatoes having a tendency to darken caused color improvement. However, none of the investigators recommended this procedure because potatoes thus prepared did not cook tender. In preliminary tests comparing recommended cooking methods (acid treatment, soaking in water, cooking in milk) acid was shown to be by far the most effective in improving the appearance of potatoes that discolored.

This study was undertaken (*1*) to observe the color, after boiling, of varieties of potatoes commonly grown in Wisconsin and of some newer varieties after various intervals of storage, and (*2*) to determine the effect of various acid treatments on color, flavor, and texture of cooked potatoes.

PROCEDURE

SELECTION OF VARIETIES

The ten varieties chosen for preliminary comparisons included those which Rieman, Tottingham, and McFarlane (*15*) and Tottingham et al. (*24*) classified as having a considerable tendency to blacken as well as some of those that seldom darken. These workers reported that Chippewa, Triumph, or Sebago potatoes rarely cook black regardless of growing conditions, but that Rural New Yorkers and Irish Cobbler often do cook black. Of the samples examined these varieties in 1945 rated for color as follows:

Good	*Fair*	*Poor*
Chippewa	Irish Cobbler	Erie
Sebago	Red Warba	Katahdin
Triumph	Russet Rural	Menomonie
	White Rural	

ENVIRONMENTAL CONTROL

To reduce possible differences in the blackening reaction that might be the result of soil or weather conditions, all potatoes used in any comparative tests were grown in the same experiment station fields. Several investigators have observed that environmental factors influence blackening in potatoes. They are: Tottingham, Nagy, and Ross (*23*), Cowie (*4*), Nash (*11*), Nash and Smith (*12*), Robinson (*17*), Smith, Nash, and Dittman (*20*), Rieman, Tottingham, and McFarlane (*15*), Tottingham et al. (*24*), Wager (*25*) (*26*), Bandemer, Schaible, and Wheeler (*1*).

All potatoes were graded and stored in the cold room of the horticulture department at the Wisconsin station at 38°–42° F. Samples were removed from storage at regular intervals and held at room temperature 10 to 14 days before the cooking tests.

SAMPLING

Previous observations disclosed that darkening in a given variety varies considerably from tuber to tuber. To obtain potatoes that were representative of each variety and as nearly identical as possible, sampling was carefully controlled. For each cooking test, tubers were selected for their uniformity in size and freedom from defects and injuries. Six to 10 tubers were used in most of the observations. Potatoes were cut in equal segments (halves, quarters, or sixths, depending upon the number of tests in a series). For each series, potato segments were distributed in a regular sequence to secure samples that were comparable.

COOKING AND EVALUATING

In a single series all samples were boiled in identical saucepans for the same length of time. After draining, the potatoes were exposed to the air 1 hour at room temperature to permit maximum development of color before color ratings were made. The pH value of the cooking water was determined at the end of the cooking period using a Beckman pH meter with a glass electrode.

Each series of cooked potatoes was scored subjectively for color, texture, and flavor by two to five judges. In addition, a judging panel of about 20 faculty members and students in advanced foods courses evaluated boiled and mashed potatoes treated with cream of tartar. This was repeated three times in order to check the conclusions of the smaller group.

Since there was a decrease in graying of the potatoes when cream of tartar was used, it may have influenced the flavor scores. A consumer preference test (279 individuals) was conducted in an effort to evaluate flavor with a minimum bias. Potatoes that did not discolor were cooked, mashed, and seasoned and then divided into two portions. One served as the control; cream of tartar was added to the other portion in the proportion of one-fourth teaspoon per pound of

peeled potatoes. Color differences between samples thus treated were too slight to be perceptible. Consumers were served two samples and asked to check their flavor preference.

RESULTS

Influence of pH on Color Scores

The effects of different acid treatments on pH values of the cooking water and the corresponding color ratings for boiled potatoes are shown in table 1. Ascorbic, citric, and tartaric acids, calcium diphosphate $CaH_4(PO_4)_2$ and cream of tartar were effective in reducing blackening, if the quantity of acid was sufficient to lower the hydrogen-ion concentration of the cooking water to pH 5 or less. This confirms the findings of Nutting and Pfund (*14*). As pH values for the cooking water decreased, numerical scores for color of boiled potatoes usually increased. When potatoes were poor and darkened severely, as in the 1946 crop, smaller amounts of acid did not always produce good

Table 1.—*Influence of pH upon color of boiled potatoes*

Year and series	Variety	Kind of cooking water or of solution	pH	Color score [1]
1945:				
1a	Erie	Distilled water	6.3	5.5
1a	Erie	Ascorbic acid solution (0.1 percent)	5.3	6.5
1945:				
1b	Erie	Distilled water	6.3	5.0
1b	Erie	Ascorbic acid solution (0.2 percent)	4.5	7.5
2a	Katahdin	Distilled water	6.6	5.5
2a	Katahdin	Ascorbic acid (0.08 percent) solution	5.4	6.5
2a	Katahdin	Ascorbic acid solution (0.16 percent)	4.9	7.5
2b	Katahdin	Distilled water	6.6	5.5
2b	Katahdin	Ascorbic acid solution (0.16 percent)	4.9	8.8
3a	Menomonie	Distilled water	6.3	5.5
3a	Menomonie	Ascorbic acid solution (0.16 percent)	4.8	8.0
3b	Menomonie	Distilled water	6.0	7.0
3b	Menomonie	Ascorbic acid solution (0.1 percent)	5.3	7.5
3b	Menomonie	Ascorbic acid solution (0.16 percent)	4.7	8.0
3c	Menomonie	Distilled water	6.1	5.0
3c	Menomonie	Ascorbic acid solution (0.2 percent)	4.7	8.0
4	Red Warba	Distilled water	6.0	7.0
4	Red Warba	Ascorbic acid solution (0.2 percent)	4.8	8.5
5a	Russet Rural	Distilled water	6.4	6.0
5a	Russet Rural	Ascorbic acid solution (0.2 percent)	4.4	9.0
2	Katahdin	Distilled water	6.6	5.5
2	Katahdin	Calcium biphosphate solution (0.16 percent) $CaH_4(PO_4)_2$.	4.5	7.5
5b	Russet Rural	Distilled water	6.4	6.5
5b	Russet Rural	Calcium biphosphate solution (0.16 percent) $CaH_4(PO_4)_2$.	5.3	7.5
5b	Russet Rural	Citric acid solution (0.2 percent)	4.2	9.0
1946:				
1	Cobblers	Tap water	8.6	5.0
1	Cobblers	Tap water plus 0.2 percent cream of tartar	4.9	6.0
1	Cobblers	Tap water plus 0.4 percent cream of tartar	4.4	6.0
2	Katahdin	Tap water	8.8	7.0
2	Katahdin	Tap water plus 0.2 percent cream of tartar	4.6	8.0
2	Katahdin	Tap water plus 0.4 percent cream of tartar	4.0	9.0
3	Menomonie	Tap water	9.0	5.0
3	Menomonie	Tap water plus 0.2 percent cream of tartar	4.6	8.0
3	Menomonie	Tap water plus 0.4 percent cream of tartar	4.4	6.0
4	Pontiac	Tap water	8.1	6.0
4	Pontiac	Tap water plus 0.2 percent cream of tartar	4.6	9.0
4	Pontiac	Tap water plus 0.4 percent cream of tartar	4.1	6.0
1947:				
1	Russet Rural	Distilled water	6.4	6.5
1	Russet Rural	Cream of tartar solution (M/20)	4.0	8.5
1	Russet Rural	Tartaric acid solution (M/20)	3.1	9.0
1	Russet Rural	Sodium potassium tartrate solution (M/20)	7.4	7.0

[1] Maximum score=10 for most desirable color.

color. When larger amounts of cream of tartar, 0.4 percent were added, a deeper yellow color developed which was considered undesirable by the judges.

The regression equation for color score (y) as a function of pH for all data, regardless of variety was found to be:

$$y=10.4-0.62 \text{ pH}$$

The regression was found to be statistically significant ($t=5.45$, 39 degrees of freedom). There is one chance in 100 that the value of t calculated from a set of data of this size would be greater than 2.71 if there were no true regression.

Effects of Cream of Tartar Treatment on Color and Texture of Boiled Potatoes

The quantity of cream of tartar and the time at which it was added markedly influenced the desirability of the potatoes, as shown in table 2. Texture scores were high if no cream of tartar was employed but color scores were low. If 1 gram cream of tartar was added at the beginning of the cooking period, as illustrated in table by the Katahdin variety of series 2, color ratings were good but textures were poor. If the same quantity of cream of tartar was added to the cooking water after potatoes had boiled 25 minutes, the acid was not detrimental to texture but this treatment was inadequate to improve color. If potatoes were boiled 20 minutes before the addition of cream of tartar, the ratings for texture were good, but color scores were fair. As the results using four different varieties show, potatoes boiled 15 minutes before the addition of cream of tartar had good texture and good color. Increasing the quantity of cream of tartar to 1½ grams or to 2 grams usually resulted in better color scores if

Table 2.—*Effect of cream of tartar treatment on color and texture of boiled potatoes*

Series No.	Variety	Cream of tartar added	Cooking time[1] before adding cream of tartar	Color score[2]	Texture score[2]
		Grams	*Minutes*		
1	Erie	None	----------	6.5	9.5
2	Katahdin	None	----------	4.5	9.0
3	Menomonie	None	----------	6.5	8.5
4	do	None	----------	6.5	9.0
5	do	None	----------	6.0	6.0
6	Pawnee	None	----------	6.0	8.5
2	Katahdin	[3] 1.0	0	8.0	6.5
2	do	1.0	25	6.5	9.0
1	Erie	1.0	20	8.0	9.5
7	do	1.0	20	8.0	9.0
5	Menomonie	1.0	20	7.0	8.0
1	Erie	1.0	15	9.5	8.5
2	Katahdin	1.0	15	7.5	8.5
3	Menomonie	1.0	15	9.0	8.0
4	do	1.0	15	8.5	8.0
5	Pawnee	1.0	15	8.0	8.0
3	Menomonie	1.5	15	9.5	8.5
4	do	1.5	15	9.5	8.5
3	do	2.0	15	10.0	7.0
4	do	2.0	15	10.0	7.0
5	do	2.0	25	8.0	8.0

[1] Total cooking time, 30 minutes.
[2] Maximum score for most desirable color or texture is 10.
[3] 1 gram of cream of tartar to 500 ml. of water equals 0.2 percent.

the potatoes had a marked tendency to darken, but the detrimental influence of acid upon texture was greater.

Photographs of Pontiac potatoes (plate 1) show the regions most affected by blackening as well as the contrast between treated and untreated sections, comparable in every detail.

Effects of Cream of Tartar on Flavor

Both the ratings by a small group of judges of flavor and scorings for color by a panel of 20 make it clear that adding cream of tartar to the cooking water reduced discoloration of potatoes, and increased the color score. Flavor is the next question that must be considered. The distribution bars (fig. 1) show the range for color and flavor scores of boiled potatoes both with and without cream of tartar treatment. Mean values were:

	Color	*Flavor*
Controls	5. 2	7. 9
Treated	6. 9	7. 6

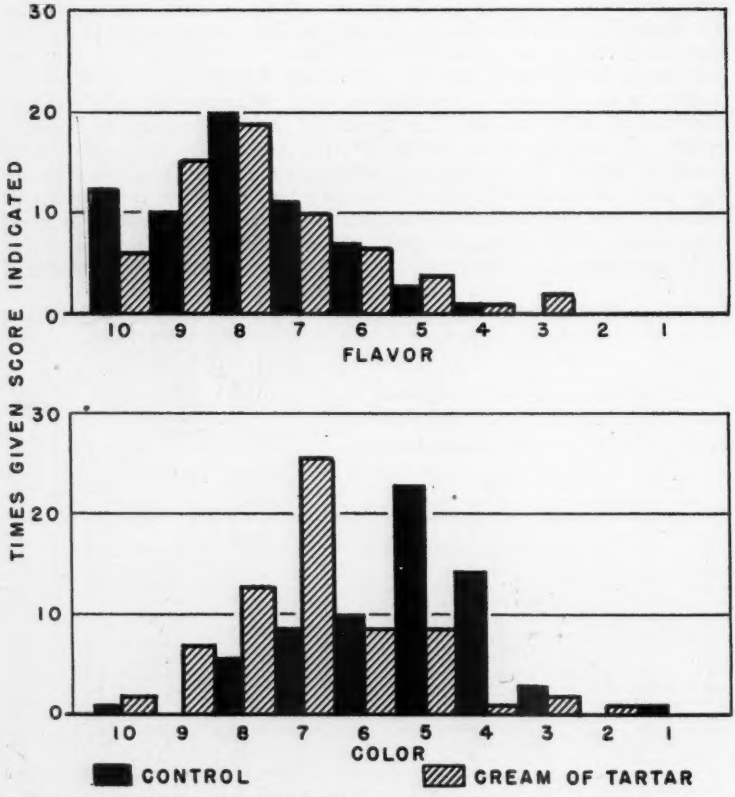
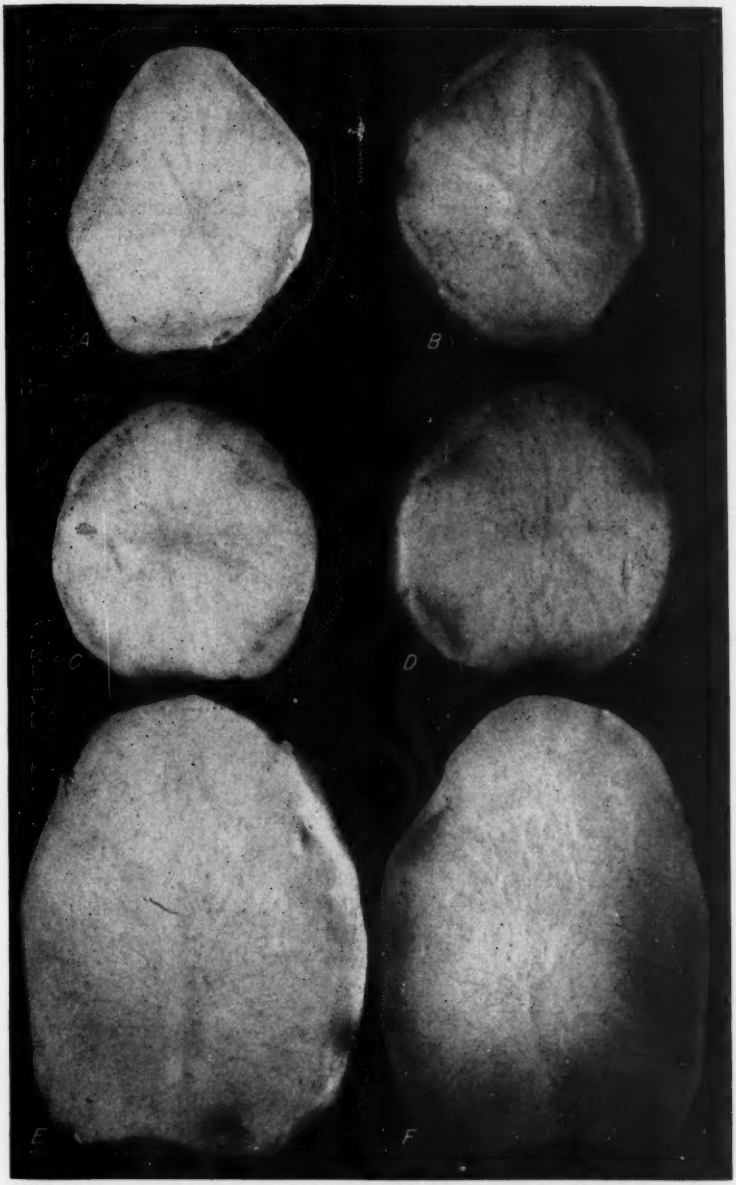


Figure 1.—Distributions of 64 flavor and 70 color scores of boiled potatoes, with and without cream of tartar.



Pieces of Pontiac potatoes. Left, treated and white; right, untreated and darkened. *A–D*, matching faces of transverse and adjacent stem-end slices. *A* and *C*, cooked in water plus cream of tartar, pH 4.2; *B* and *D*, cooked in water of pH 8.5. *E* and *F*, matching halves cut longitudinally. *E*, boiled in water plus cream of tartar, pH 4.6; *F*, boiled in water of pH 8.6.

Figure 1 shows that the distributions for scores on flavor of boiled potatoes were similar except for the greater frequency of 8 for both the control and the treated samples. The distributions for color scores reached a maximum of 5 for the control and 7 for the treated samples.

The results for color and flavor judging for mashed potatoes are given in figure 2. The distribution shows that color scores were definitely higher in potatoes which had cream of tartar added when mashing. Flavor evaluations varied widely, but the mean value for mashed potatoes to which cream of tartar was added was low. The means were:

	Color	*Flavor*
Controls	7. 1	8. 2
Treated	9. 4	6. 2

These results indicated that cream of tartar improved the color of mashed potatoes but for a number of the judges flavor was impaired.

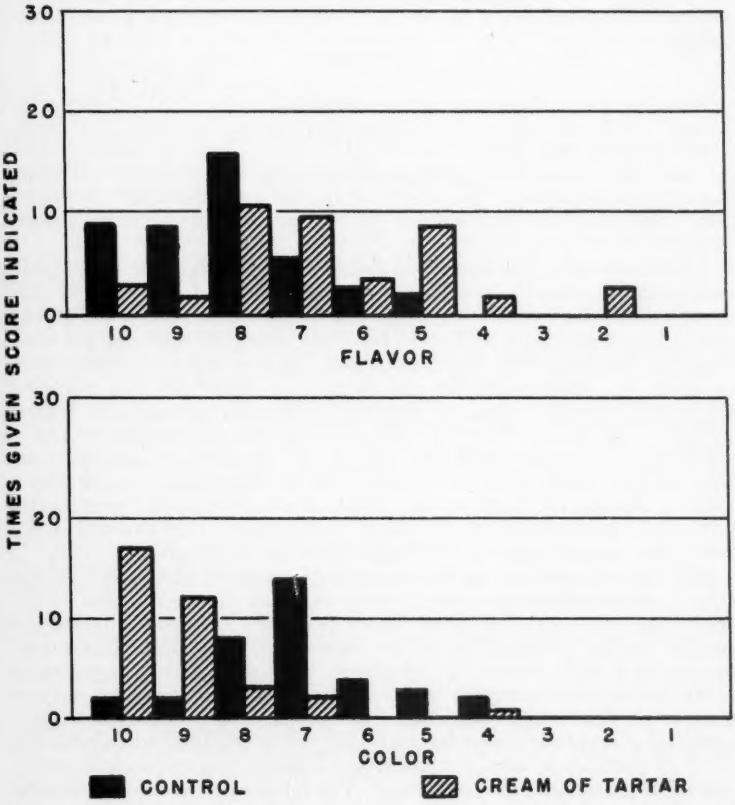


FIGURE 2.—Distribution of 35 flavor and color scores of mashed potatoes, with and without cream of tartar.

No salt or seasonings had been used in either case. Because there was such a wide range in the flavor scores and the color gave a clue to treatment, the question of flavor was investigated further.

In the consumer preference study on seasoned mashed potatoes additional information was secured concerning the effects of cream of tartar on flavor. The results and the statistical analysis follow:

Total number of individuals:	*Number preferring cream of tartar sample:*	*Number preferring sample without cream of tartar:*
279	161	118

$\chi = 6.627$

χ^2 at 5 percent level for 1 d. f. = 3,841; at 1-percent level = 6.635.

These data show that the preferences differ significantly from an expected ratio of 50:50 and approach the value at the 1-percent level.

Figure 2 shows that the greatest frequency of the distributions for scores on flavor of mashed potatoes were, for the controls, at 10, 9, and 8, and for the treated samples, at 8, 7, and 5. Similarly for color scores it was at 8 and 7 for the controls and 10 and 9 for the treated samples.

DISCUSSION

The favorable effect of acetic, ascorbic, citric, hydrochloric, and tartaric acids, as well as lemon juice, calcium diphosphate, and potassium tartrate was exerted on the pH only. This was demonstrated by the fact that when each acid medium was rendered alkaline, darkening developed. The factor or factors responsible for discoloration thus behaved reversibly with change in hydrogen-ion concentration.

That darkening has some relationship to tyrosinase or some labile substance activated by tyrosinase was indicated by Clagett and Tottingham (*3*), Hansen (*7*), Nagy and Tottingham (*10*), and Tottingham, Nagy, and Ross (*23*). It has been reported that certain compounds, among them boric acid and benzoic acid, combine with catechol or other phenolic compounds to interrupt the catalytic activity of pure tyrosinase (Behm and Nelson (*2*), Ludwig and Nelson (*9*) and Nelson and Dawson (*13*)). In this connection the results at the Wisconsin station with these two acids in native potato tissue are interesting. Boric acid or benzoic acid in the cooking water had a marked inhibitory effect on the development of blackening of boiled potatoes. In contrast to the other acids tested, this effect was not reversible with changes in hydrogen-ion concentration.

Although treatments with several acids reduced the extent of discoloration in cooked potatoes with a tendency to darken, few of the treatments were suitable for culinary purposes. Cream of tartar has certain distinct advantages in this respect. It is available, harmless, inexpensive, and reasonably effective. Not only does the action of cream of tartar compare favorably with other acids, but it is easy to measure. For boiled potatoes, the proportion of one-fourth teaspoonful of cream of tartar for each pint of water added when potatoes are half cooked, is best when all palatability factors, such as color, flavor, and texture are considered. For mashed potatoes, one-fourth teaspoonsful of cream of tartar per pound of peeled potatoes may be added at the time of mashing with such other seasonings as salt,

milk, and butter. The favorable effect upon color is thus obtained without any detrimental influence of acid upon texture and ease in mashing.

SUMMARY AND CONCLUSIONS

This study of the effect of acids upon color of potatoes susceptible to darkening confirms the work of others that there is a direct and reversible relationship between hydrogen-ion concentration and degree of darkening.

For two acids, boric and benzoic, some additional factor is probably involved since their effect could not be reversed by simply changing hydrogen-ion concentration.

Food tasting panels were employed to determine the practical application and consumer acceptability of potatoes treated with cream of tartar. Color scores for potatoes that normally cooked dark were significantly higher when the cream of tartar treatment was used than it was in untreated samples. The undesirable effect of acid upon texture of potatoes was reduced if the cream of tartar was added at the mid-point of cooking for boiled potatoes, or at the time of mashing. Flavor acceptability tests in which 279 individuals participated showed that a statistically significant number preferred the flavor of mashed potatoes to which cream of tartar had been added with the other seasonings such as salt, butter, and milk.

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THE OCCURRENCE OF HYPODERMA LARVAE IN THE SPINAL CANAL OF CATTLE [1]

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INTRODUCTION

Little is known of the migratory route of *Hypoderma bovis* (L.) larvae in cattle. The absence of larval traces from the point of skin penetration to the various positions from which the larvae have been recovered has made this problem difficult. The occurrence of *Hypoderma* larvae in the spinal canal of cattle has been recorded on numerous occasions since its discovery by Hinrichsen (*4*) in 1888 in Germany. However, few observations have been made in North America. The purpose of this paper is to present our observations on *Hypoderma* larvae in the spinal canal of cattle killed in Wisconsin.

Prior to the work of Laake (*13*) on the differentiation of *Hypoderma lineatum* (Villers) and *H. bovis*, investigators were uncertain about the identity of the first-stage larvae. As suggested by Bishopp et al. (*1*), many of the previous reports dealing with the occurrence of *H. bovis* in the esophageal region must be considered in error. Consequently, the identity of larvae in the spinal canal mentioned in reports prior to 1921 is questionable.

Hinrichsen (*4*, *5*, *6*) apparently found the first stage larvae of *Hypoderma bovis* in the spinal canal. He reported finding up to 20 larvae in a single animal. Of 25 animals examined, 40 percent had larvae in the spinal canal. A later examination of 14 animals revealed that 28 percent were infested. The larvae appeared to be numerous between December and March. Horne (*7*), unaware of Hinrichsen's earlier work, encountered, in the spinal canal of Norwegian cattle, *Hypoderma* larvae which he assumed to be *H. bovis*. Ruser (*7*, *8*) in Germany reported the presence of as many as 20 larvae in the spinal canal of 1 animal. Schneidemühl (*20*) frequently discovered larvae in the epidural fatty tissue between February and April. Koorevaar (*10*, *11*) in Holland also recorded *Hypoderma* larvae in the spinal canal of cattle; in 1 young animal 57 larvae were collected. In experimental transplantation of 26 larvae in a dog, 2 larvae were recovered 14 days later from the spinal canal. Koch (*9*) in 1903 observed larvae in the spinal canal of cattle between the months of December to March in Denmark; similar results were obtained by Jost (*8*) in Germany. Peter (*15*) working in Germany concluded that the number of larvae in the spinal canal was usually small, but in the case of 1 bull 62 larvae were observed.

The 30 larvae collected by Bishopp et al. (*1*) from the spinal canal of New York cattle where both species are present, were identified as

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Hypoderma bovis, while only a single specimen was found in Texas cattle where only *H. lineatum* occurs. Mote (*14*) in Ohio reported 6 *H. bovis* larvae in the spinal canal of a cow. In France, Popesco-Baran (*16*) found 15 larvae in the spinal canal of an 8-to-10-month-old calf. Schmid (*19*) reported that 4 of 68 animals in Germany were infested. The following year 10 larvae were located in the spinal canal of a bull. Soni (*21*) recorded the occurrence of larval *H. lineatum* in the spinal canal of cattle in India. Gebauer (*3*), working in Germany, reported finding larval traces in the epineural connective tissue of 2 calves. Three *H. bovis* larvae were found associated with the characteristic greenish extraneous material present along the nerve trunks from the foot to the spinal canal. The more than 20 larvae removed from the spinal canals were *H. bovis*. The most comprehensive work to date was done by Eichler (*2*) in 1941 in Germany. All the larvae identified of the 189 collected from the spinal canal were *H. bovis*. He also identified as *H. bovis* 11 of 36 larvae, which were collected by Staack in 1938. Kurtpinar (*12*), working in Turkey, where only *H. bovis* is found, frequently observed the larvae in the canalis vertebralis.

MATERIALS AND METHODS

The fatty connective tissue which lies between the periosteum and dura mater in the spinal canal was removed from cattle slaughtered at a local abattoir and taken to the laboratory for examination. Material was collected on the cattle-killing floor; facilities were made available through the courtesy of the Oscar Mayer Packing Co.,

TABLE 1.—*Summary of collections of* Hypoderma *larvae from the spinal canals of 293 cattle*

Period of examination	Spinal canals examined	Spinal canals infested	Larvae present		Average larvae per canal	Size range of larvae		Average length of larvae	
			H. bovis	*H. lineatum*		*H. bovis*	*H. lineatum*	*H. bovis*	*H. lineatum*
	Number	*Number*	*Number*	*Number*	*Number*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*
1947									
February	20	5	11		2.2	11.0–14.0		12.7	
March	95	9	21		2.3	11.0–16.0		13.6	
April	[1] 150	4	4		1.0	14.0–15.0		14.7	
May	[1] 170	2	5		2.5	15.0–16.5		15.6	
June	[1] 185	0							
July	70	0							
August	180	0							
September	150	0							
October	140	0							
November	185	5	33		6.6	5.0–11.0		8.1	
December	190	6	10	2	1.7	7.0–14.5	14.0–14.5	9.3	14.2
1948									
January	185	41	153		3.7	5.0–16.0		11.7	
February	275	107	497	5	4.6	7.0–17.0	14.0–15.5	12.2	14.7
March	328	76	146		1.9	8.0–16.0		12.9	
April	165	22	58		2.6	8.5–16.0		12.4	
May	65	1	1		1.0	15.0		15.0	
June	105	0							
July	50	0							
August	255	0							
September	250	1	1		1.0	7.5		7.5	
October	335	14	35		2.5	5.5–13.0		8.1	

[1] Approximate number of spinal canals examined.

Madison, Wis. Samples of tissue from each spinal canal were macerated with forceps and examined closely for the presence of larvae. These were examined microscopically either as living or in the preserved state.

Identifications were based on differences in the cephalopharyngeal skeleton of the first stage larvae (*13*). The results of this investigation are shown in table 1.

RESULTS

Of 982 larvae removed from 293 infested spinal canals, 975 were identified as *Hypoderma bovis* and 7 as *H. lineatum*. The maximum number of larvae removed from a single infected spinal canal was 21. The length of the larvae ranged from 5.0 to 17.0 mm., and a progressive increase in the average length was noted from September (7.5 mm.) to May (15.6 mm.).

Both species of the warble fly, *Hypoderma bovis* and *H. lineatum*, were present in this area (Wisconsin), but *H. bovis* was found to be the most abundant. However, this work included examinations of animals from other areas where *H. lineatum* is more prevalent.

An examination of a comparable group of animals during the same period indicated a prevalence of *Hypoderma* larvae in the esophageal region. Of 982 larvae removed from infested esophagi, 981 were *H. lineatum* and only 1 was *H. bovis*.

SUMMARY

Our observations on the occurrence of *Hypoderma bovis* in the spinal canal of cattle indicated a distinct difference in the migration route followed by the larvae of the two species. Only the larvae of *H. bovis* occurred regularly in the spinal canal whereas those of *H. lineatum* were rarely found in the spinal canal. On the other hand, only *H. lineatum* occurred regularly in the esophageal region. The increase in the average length of the larvae and in the number collected between September and June indicated that the larvae gradually accumulate in this region prior to appearing in the subdermal regions of the back. This occurrence corresponds with the well-known accumulation of *H. lineatum* larvae in the submucosa of the esophagus.

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TOXICITY STUDIES OF DDT-SABADILLA MIXTURES WITH EVIDENCE OF SYNERGISTIC ACTION[1]

By J. T. MEDLER, *assistant professor*, and H. E. THOMPSON, *research assistant, in agronomy and economic entomology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

Experiments, made in Wisconsin during the summer of 1946 with insecticides for the control of insects affecting alfalfa seed production and published by Medler and Scholl,[2] showed that a dust mixture of 2.5 percent DDT and 5 percent sabadilla (lime-activated) gave higher yields of alfalfa seed than did 5 percent DDT or 10 percent sabadilla. Since synergistic action was suggested by these results, further tests were made in the laboratory with DDT and sabadilla in order to evaluate the relative toxicities of various mixtures.

MATERIALS AND METHODS

The majority of tests were made with 5-day-old houseflies, *Musca domestica* L., reared on a synthetic medium as specified by the Peet-Grady method. Additional tests were made when possible with tarnished plant bugs, *Lygus oblineatus* (Say) and alfalfa plant bugs, *Adelphocoris lineolatus* (Goeze), collected from alfalfa fields. DuPont 50 percent DDT and McConnon 50 percent sabadilla (lime-activated) wettable powders were prepared alone or in mixtures in concentrations of 4.8, 2.4, 1.2, and 0.6 gm. per liter.[3] These concentrations were estimated to be comparable with 2, 1, ½, and ¼ pounds of toxicant in 100 gallons of water.

The spray-tower apparatus described by Waters[4] and a petri-dish testing method, which have been adapted and improved for rapid DDT testing at the University of Wisconsin, were used for the experiments. Suspensions of the insecticidal mixtures were sprayed at a pressure of 10 pounds into a vertical cylinder 8 inches in diameter and 3 feet high. An open petri dish was exposed to the spray at the bottom of the cylinder for 5 seconds. The toxicity of the insecticide deposited in the petri dish was evaluated on the basis of the time required for a 50 percent knock-down of 10–20 insects introduced into the dish and covered with a clean glass plate. The petri dishes were tested with houseflies in eight replicates within 2 hours after spraying for the

[1] Received for publication January 24, 1949. Supported in part by The Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

[2] MEDLER, J. T., and SCHOLL, J. M. CONTROL OF INSECTS AFFECTING ALFALFA SEED PRODUCTION IN WISCONSIN. Jour. Econ. Ent. 40: 579–518. 1947.

[3] All data in this paper are given as the amount of 50-percent wettable powder used. The amount of actual toxicant is one-half of these concentrations.

[4] WATERS H. A. LABORATORY PROCEDURES IN STUDIES OF THE CHEMICAL CONTROL OF INSECTS. Amer. Assoc. Adv. Sci. 1943, pp. 115–123.

initial action and in four replicates at 5-, 10-, 20-, and 30-day intervals for the residual action. The tarnished plant bugs and alfalfa plant bugs were tested in four replicates at the initial test and after 1 week.

RESULTS

The results of the tests with houseflies are given in table 1, and with the tarnished plant bugs and alfalfa plant bugs in table 2. These data were analyzed for variance by statistical methods, and significant differences were found between the treatments.[5]

TABLE 1.—*Average times for a 50-percent knock-down of houseflies when exposed to DDT-sabadilla mixtures on petri dishes*

Mixture in grams per liter		Interval				
50 percent DDT wettable powder	50 percent sabadilla wettable powder	Initial [1]	5 days [2]	10 days [2]	20 days [2]	30 days [2]
		Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*
0.6		28.5	[3]	[3]	[3]	[4]
.6	0.6	27.8	[3]	[3]	[3]	[4]
.6	1.2	23.9	46.5	88.8	[3]	[4]
.6	2.4	21.6	38.0	89.8	[3]	[4]
.6	4.8	19.0	53.5	[3]	[3]	[4]
1.2		20.9	40.0	68.3	[3]	[4]
1.2	.6	19.4	48.3	90.0	[3]	[4]
1.2	1.2	18.6	27.3	48.0	[3]	[4]
1.2	2.4	16.0	50.0	105.5	[3]	[4]
1.2	4.8	12.8	48.0	148.5	[3]	[4]
2.4		16.8	25.3	24.3	21.3	18.0
2.4	.6	16.1	24.3	23.8	25.5	17.0
2.4	1.2	15.1	24.8	31.3	27.3	18.3
2.4	2.4	13.8	27.3	20.5	24.0	17.5
2.4	4.8	12.4	18.5	17.3	17.5	19.3
4.8		15.1	21.3	18.3	15.5	13.5
4.8	.6	14.5	17.3	16.8	13.8	12.8
4.8	1.2	13.4	17.8	17.3	15.3	11.5
4.8	2.4	13.5	18.5	14.8	16.0	13.3
4.8	4.8	13.1	20.3	16.3	16.0	14.0
	.6	[3]	[4]	[4]	[4]	[4]
	1.2	[3]	[4]	[4]	[4]	[4]
	2.4	[3]	[4]	[4]	[4]	[4]
	4.8	[3]	[4]	[4]	[4]	[4]

[1] 8 replications.
[2] 4 replications.
[3] 50-percent knock-down not reached in 180 minutes.
[4] No test made.

The housefly experiments were calculated as regressions of dosage of insecticidal mixture on 50-percent knock-down times. The regression lines were found to be linear when both variables were converted to logs. These regression lines are shown in figure 1, the concentration of DDT being the independent variable. The lines representing DDT alone and DDT plus the four different concentrations of sabadilla show that the toxicity of DDT is proportionally increased by the addition of sabadilla.

[5] Acknowledgment is made to The Computing Service of the University of Wisconsin for analysis of the data.

TABLE 2.—*Average times for a 50-percent knock-down of tarnished plant bugs and alfalfa plant bugs when exposed to DDT-sabadilla mixtures on petri dishes*

Insect	Mixture in grams per liter		Interval	
	50 percent DDT wettable powder	50 percent sabadilla wettable powder	Initial [1]	1 week [1]
Tarnished plant bugs:			*Minutes*	*Minutes*
Adults	1.2	1.2	20.5	200
	2.4		127.5	182.5
	2.4	1.2	18	[3]
	4.8		139	146
	4.8	.6	21.5	76.5
	4.8	1.2	41.5	[3]
	4.8	2.4	11	[3]
		.6	[2]	[3]
		1.2	28.0	[3]
		2.4	10.5	[3]
Nymphs	1.2		152.5	[3]
	2.4		71	[3]
	4.8		41.5	66.5
Alfalfa plant bug, adults	2.4	1.2	69.5	[3]

[1] 4 replications.
[2] Not tested.
[3] 50 percent not reached in 4 hours.

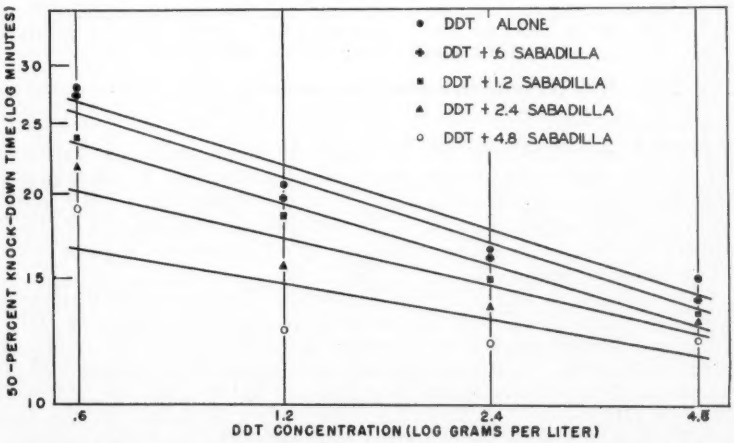

FIGURE 1.—Fifty-percent knock-down times at initial exposures of houseflies to DDT and DDT-sabadilla mixtures.

Regressions of initial tests are plotted in figure 2 using the concentration of total insecticide in each mixture as the independent variable. The regression lines based upon actual values were linear. The slopes of the lines show that more pronounced effects are obtained with mixtures of DDT in concentrations of 0.6 and 1.2 gm. per liter. The major toxicant in each combination is DDT, e. g., at 3.6 the addition of 1.2 sabadilla to 2.4 DDT is more effective than the addition of 2.4 sabadilla to 1.2 DDT. However, the lines connecting 1.2 and

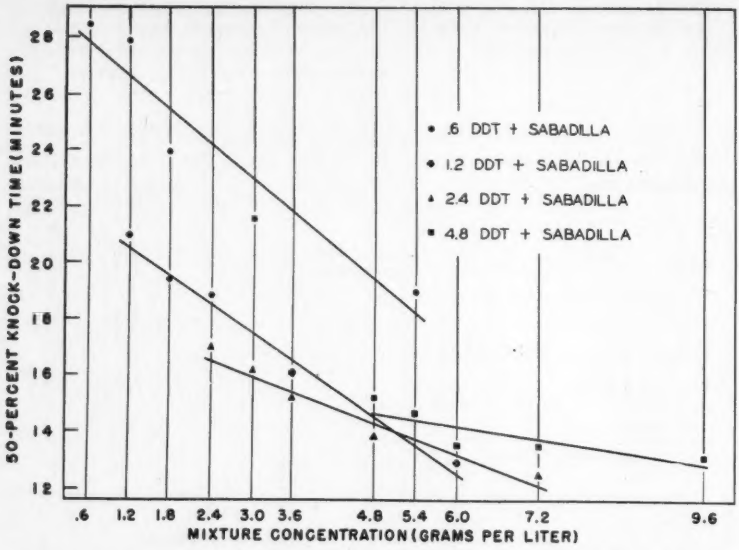

FIGURE 2.—Fifty-percent knock-down times at initial exposures of houseflies to DDT and DDT-sabadilla mixtures.

2.4 DDT concentrations are in relatively close proximity between 3.6 and 6.0, and a cross-over occurs between 4.8 and 5.4, which suggests that the addition of sabadilla to DDT is more practical in this range than at other concentrations. The addition of sabadilla to 4.8 DDT apparently does not accelerate the toxicity of the mixture in proportion to the amount used.

Data plotted in figure 3 show the residual action of the mixtures. The 50-percent knock-down times of the four concentrations of DDT alone are plotted against the corresponding DDT-sabadilla mixtures in a simple curve. The latter points were obtained from data in table 1 by averaging the times of the four different DDT-sabadilla mixtures at each DDT level. A loss of residual effect is shown for the 0.6 DDT alone at 5 days, yet 0.6 DDT plus sabadilla gives knock-downs at 10 days. The loss of residual effect of 1.2 DDT and DDT plus sabadilla mixtures both take place after 10 days. The residual action of 2.4 and 4.8 DDT concentrations at the end of 30 days is practically the same as it is initially, and on the average it appears that no significant increased toxicities result from the addition of sabadilla beyond the 10-day interval. The apparently better knock-down of the 4.8 DDT at 30 days than initially perhaps may be explained by a gradual dissipation of some masking agent that might be in the "inert" part of the wettable powder.

The tests with tarnished plant bugs and alfalfa plant bugs were made during a brief period in August when populations were high enough in the field to provide an adequate number of test insects.

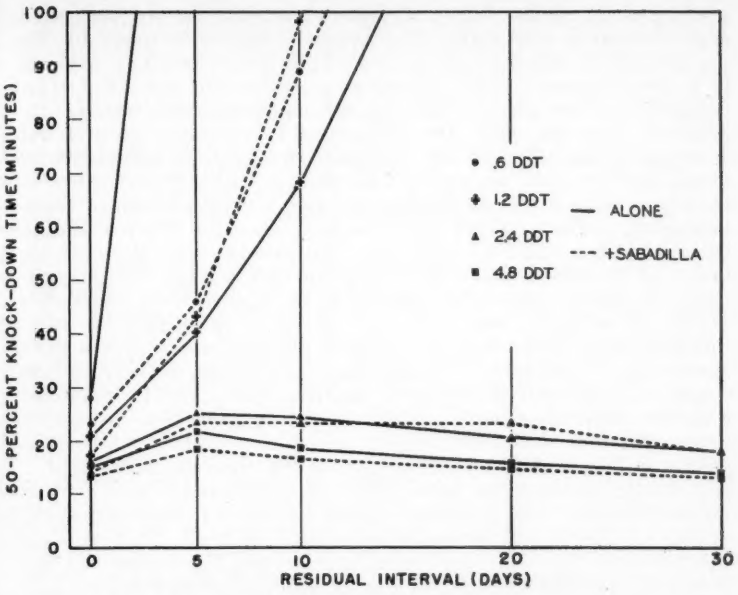

FIGURE 3.—Fifty-percent knock-down times at interval exposures of houseflies to DDT and DDT-sabadilla mixtures.

Therefore, only limited observations were possible. An inspection of the data in table 2 shows that sabadilla is the major toxicant of the insecticidal mixture. This is a reversal of the action of DDT-sabadilla mixtures on houseflies. A 2.4 concentration of sabadilla knocks down the tarnished plant bug faster than 2.4 DDT knocks down a housefly. The sabadilla loses most of its effectiveness in a week's time, but data show that the 4.8 DDT plus 0.6 sabadilla mixture gives a quicker knock-down at 1 week than 4.8 DDT alone and suggest some residual effect of sabadilla at certain concentrations.

Data in table 2 show also that equivalent concentrations of DDT are more toxic to nymphs than adults of tarnished plant bugs, and that the alfalfa plant bug is more resistant to a 2.4 DDT plus 1.2 sabadilla mixture than is the tarnished plant bug.

DISCUSSION

Wadley[6] defined synergism as a joint action of two materials, such that the total effect is greater than the sum of the two effects when each is used alone, and stated that its determination requires some estimate of similar joint effect inferred from the action of each ingredient used alone. Previous investigations depended largely upon

[6] WADLEY, F. M. THE EVIDENCE REQUIRED TO SHOW SYNERGISTIC ACTION OF INSECTICIDES AND A SHORT CUT IN ANALYSIS. U. S. Bur. Ent. and Plant Quar. ET-223, 6 pp. 1945.

a comparison of the slopes of dosage-mortality curves between 40 and 95 percent mortality. The present analysis is made by comparisons of 50-percent knock-down times, which are not applicable to a dosage-mortality curve because a 100-percent mortality always results from the use of DDT at the concentrations tested. It is believed, however, that the 50-percent knock-down gives a valid estimate of the effect of the combination and each ingredient used alone, since the time required for an effect is probably correlated with toxicity. Should this correlation be true, the data obtained suggest synergistic action of DDT and sabadilla, since the effect of the mixtures is greater than the joint effect predictable from the individual action of the ingredients. With tarnished plant bugs, the synergistic action is clearly shown by comparing the knock-down times of 2.4 DDT, 2.4 sabadilla, and a mixture of 1.2 of each, 127, 10, and 20 minutes, respectively. Since 1.2 sabadilla used alone gave a knock-down time of 28 minutes, it is seen that the addition of DDT increased toxicity significantly, for it can be assumed that 1.2 DDT would have a slower action than would 2.4 DDT. With houseflies, the evidence is not clear-cut, since a 50-percent knock-down could not be obtained with sabadilla within 3 hours at any of the concentrations used. If this evidence is used as an estimate of the probable action of that ingredient alone, the increased toxicities obtained when sabadilla is added to DDT are probably synergistic action. This is clearly demonstrated by the almost equal knock-down times shown in figure 2 at three combinations of 4.8 gm. of mixture concentration.

SUMMARY AND CONCLUSIONS

DDT and sabadilla mixtures were sprayed on petri dishes, and the residual films were tested for relative toxicity by measuring the time required for a 50-percent knock-down of houseflies and of tarnished plant bugs and alfalfa plant bugs. The toxic action of the mixtures was tested at an initial period and at 5-, 10-, 20-, and 30-day intervals.

Data obtained show that a synergistic relationship exists with sabadilla and DDT mixtures, since the effect of the mixtures was greater than the sum of the individual effects of the two components.

A loss of residual toxicity was observed for low concentrations of DDT, but high concentrations gave practically the same knock-down times at the 30-day test as the initial test. The data show that sabadilla gives a residual effect in some mixtures at 5- and 10-day intervals.

It is hypothesized that a masking effect of DDT by "inert" particles may have been present at certain conventrations where the synergism was not clearly demonstrated.

The laboratory findings may be utilized in a practical way for insecticides used in the field. Formulas can be prepared with sabadilla that give high initial toxicity with decreased amounts of DDT. This may be particularly important when DDT is used for the treatment of forage crops where the residue hazard may be present.

A VIRUS DISEASE OF WATERMELON IN WISCONSIN INCITED BY THE TOBACCO RINGSPOT VIRUS[1]

By GLENN S. POUND

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INTRODUCTION

In August 1946, marked viruslike symptoms were observed on watermelon (*Citrullus vulgaris* Schrad.) and muskmelon (*Cucumis melo* L.) plants in a field at Merrimac, Wis. Inoculations from infected plants to tobacco (*Nicotiana tabacum* L.), cucumber (*Cucumis sativus L.*), and watermelon demonstrated that the disease was of virus origin. Isolates from watermelon and muskmelon were identical, and one from watermelon was chosen for further study. In 1947 plantings in the same area were surveyed; and again the same virus was recovered from both watermelon and muskmelon, although the incidence was lower than in 1946. In 1948 the disease appeared in much greater proportion than in the two preceding years, affecting about 10 percent of the watermelon plants. Considerable loss resulted. This paper is a description of symptoms of the disease in the field and on certain host plants in the greenhouse, as well as a description and identification of the virus. A preliminary report has already been made (*9*).[3]

MATERIALS AND METHODS

The virus studied was obtained from a single watermelon plant growing at Merrimac, Wis. Stock cultures were kept in tobacco in aphid-proof cages. Inoculations were made by the rubbing method on plants previously sprinkled with carborundum. Greenhouses were fumigated frequently for aphid control. Air temperatures ranged 75° to 80° F. except where otherwise stated. In the host-range study, at least five plants were used in each test inoculation. From each host inoculated, recovery inoculations were made to a suitable test plant to prove the presence or the absence of the virus. Property studies were made according to methods previously detailed (12).

[1] Received for publication January 31, 1949.

[2] Appreciation is expressed to Eugene Herrling for making the photographs used in this manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 658.

EXPERIMENTAL RESULTS

SYMPTOMS AND HOST RANGE

Field Symptoms on Muskmelon and Watermelon

Naturally infected muskmelon plants showed marked stunting and a yellow-green leaf mottle accompanied by leaf malformation. Fruit set and size were greatly reduced. Symptoms were indistinguishable from those caused by viruses of the *Cucumber virus* 1 group, except that after several weeks, symptoms produced by the watermelon virus became considerably masked. Infected watermelon plants were severely stunted and chlorotic, and they produced no marketable fruits. Fruits which set were warty and occasionally exuded small drops of a viscous liquid at necrotic points on the surface. A diagnostic feature of infected vines was the upright position of distal ends of runners compared with the nearly prostrate position of healthy plants. This characteristic was discernible from a considerable distance and provided an easy way of locating infected vines. Leaf symptoms on watermelon consisted of a coarse mottle accompanied by irregular black, necrotic lesions (fig. 1, *A*). Affected leaves, when tattered and necrotic, somewhat resembled symptoms induced by *Colletotrichum lagenarium* (Pass.) E. & H. Internodes were markedly shortened, producing a compact, bunchy growth (fig. 2). Infected leaves and stems were exceedingly brittle. Infected plants showed some recovery toward the end of the season, but symptoms were much more persistent than for muskmelon.

Greenhouse Reaction on Tobacco

Local symptoms on inoculated leaves of Havana tobacco appeared first as faint chlorotic rings and ringspots but later became etched or necrotic, depending upon the temperature. Systemic symptoms appeared as concentric chlorotic rings and ringspots which often were conspicuously yellow and numerous enough to produce a mottled effect. Often a jagged (oak-leaf) pattern was associated with the veins (fig. 3, *B*). Finally, leaves lost the ringspot patterns and developed a conspicuous diffuse yellowing (fig. 3, *A*). Eventually, masking was almost complete, but yellowing was persistent on the lower leaves.

Greenhouse Reaction on Bean and Pea

Every variety of bean tested was found to be susceptible to the virus, but three did not develop pronounced systemic symptoms. The reaction was almost wholly necrotic, occasional varieties showing some mottling. Primary symptoms usually consisted of reddish-brown necrotic flecks, spots, or ringspots with occasional streaking of the veins (fig. 4, *A*). The most characteristic systemic symptom was top necrosis, a necrotic blighting of the growing tip. In practically all varieties in which systemic development occurred, the terminal growing point withered and died within 7 days after inoculation as if killed by steam (fig. 5). Occasionally plants recovered from this top necrosis and from lateral buds produced severely stunted leaves with

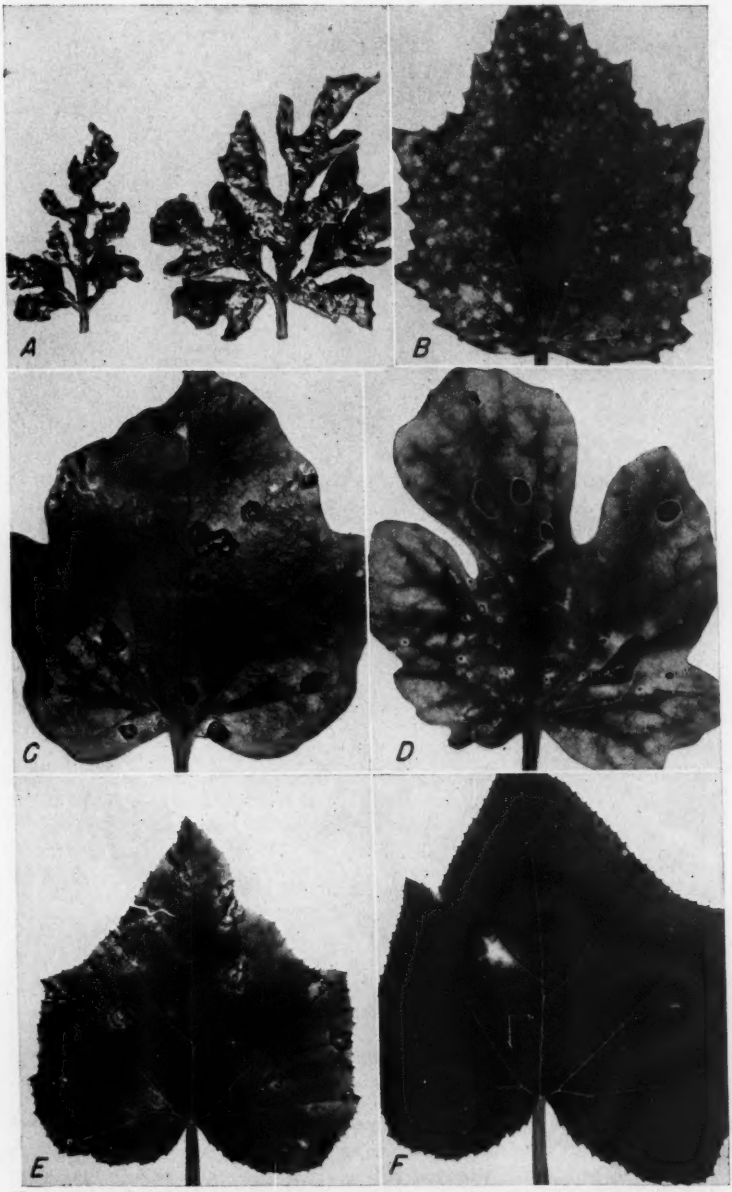

FIGURE 1.—Symptoms produced on various cucurbits by the watermelon virus. *A*, Systemic symptoms of naturally infected watermelon; *B*, systemic symptoms on inoculated cucumber; *C*, *D*, local and systemic symptoms, respectively, on inoculated watermelon; *E*, *F*, local and systemic symptoms, respectively, on inoculated Early Prolific squash.

FIGURE 2.—Portion of naturally infected watermelon vine, showing much shortened internodes.

FIGURE 3.—Leaves of Havana tobacco systemically infected with the watermelon virus. *A*, Old leaf showing persistent diffuse yellowing; *B*, young leaves showing ringspotting and oak-leaf pattern.

a coarse mottle (fig. 4, *B*). Other characteristic systemic symptoms were necrotic cankers on main stem, leaf petioles, and necrotic flecking or spotting of leaves (fig. 4, *C*). The following varieties showed systemic development as indicated by tip blight, leaf mottle, and necrosis, and necrotic cankers of petioles and stems: Florida Belle, Stringless Black Valentine, Tenderpod, Tendergreen, Bountiful, Plentiful, Keystonian, Sensation Green Pod, Sensation Refugee 1066, Striped Creaseback, Kentucky Wonder, Blue Lake, Full Measure, Rival, U. I. No. 1, and Pinto. Varieties Michelite, Robust, and Rust Resistant Kentucky Wonder showed no systemic development of tip blight or leaf necrosis but did develop occasional cankers of the main stem above the inoculated leaves. Stringless Green Refugee developed necrotic cankers of petioles and stem and slowly developing necrotic lesions of top leaves, but no tip blight. Rarely did more than one necrotic spot occur on one leaf. All bean varieties were tested at air temperatures of 16°, 20°, and 28° C. Symptom type was not changed by temperature, but incubation periods were shorter and symptoms were more severe at high temperatures.

The pea varieties Alaska, Delwiche Commando, Merit, Pride, and Prince of Wales all showed identical reactions. Inoculated leaves developed irregular necrosis; systemic symptoms were severe stunting, yellow mottling (fig. 4, *D*), and necrotic streaking of the main stem followed by death. In no case did inoculated plants survive infection.

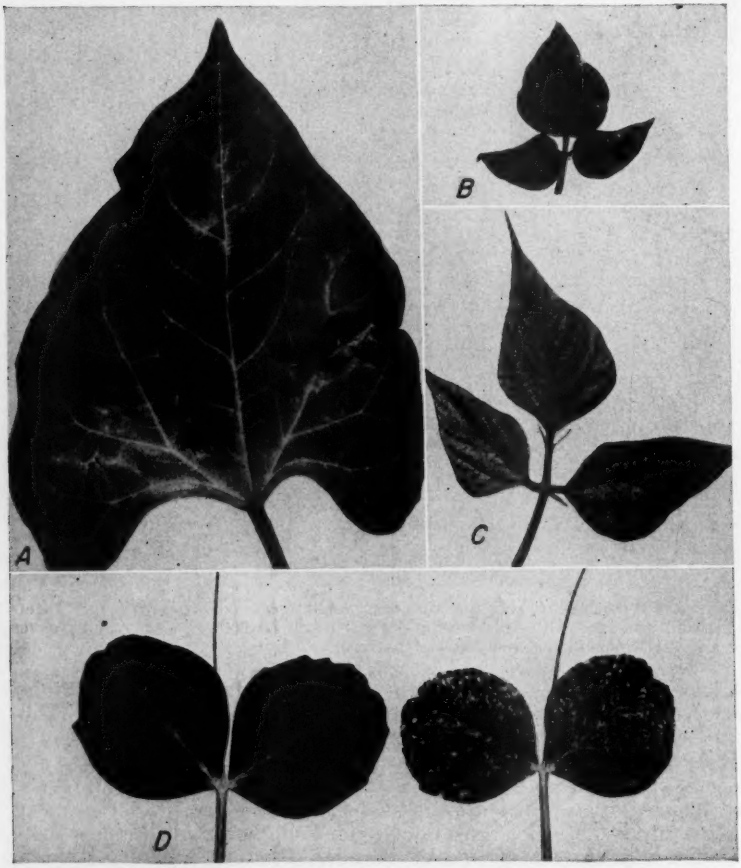

FIGURE 4.—Symptoms induced on bean and pea by the watermelon virus. *A*, local lesions on primary leaf of Pinto bean; *B*, systemic mottle; *C*, systemic necrotic flecking produced on Florida Belle bean; *D*, healthy (left) and systemically infected leaf of Alaska pea.

GREENHOUSE REACTIONS OF OTHER HOSTS TESTED

In table 1 are given the symptoms produced on selected greenhouse plants. In all cases, except where the contrary is indicated, the virus was recovered from each host which produced symptoms. The reactions on cucumber, watermelon, and squash are shown in figure 1.

EFFECT OF TEMPERATURE ON SYMPTOM EXPRESSION

The effect of air temperature on symptom development was studied on tobacco and cucumber. On cucumber, chlorotic rings were pro-

duced on inoculated cotyledons at 28° and 24°, while there was severe bleaching at 20° and 16°. Systemic symptoms developed first at 28° and last at 16°. At all temperatures, conspicuous chlorotic mottling occurred. At the high temperatures there was a marked tendency toward recovery while at low temperatures symptoms were persistent.

On tobacco, local symptoms ranged from chlorotic rings and ringspots at 28° to conspicuous necrotic rings at 16°. Systemic symptoms appeared at 28° and 24° as conspicuous rings and ringspots with considerable stunting and distortion. Chlorotic rings were gradually replaced by a diffuse yellowing which was persistent on old leaves but

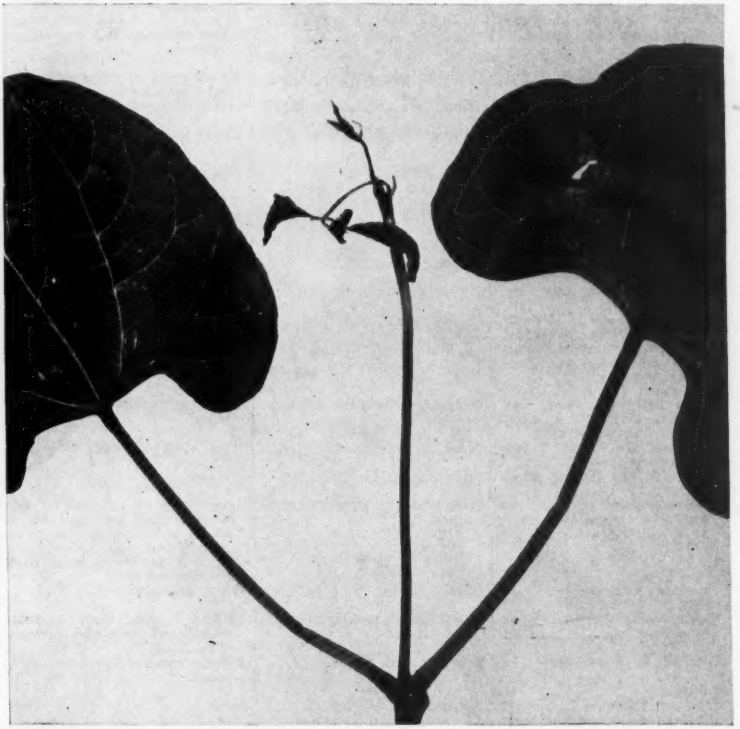

FIGURE 5.—Characteristic top necrosis induced in bean by the watermelon virus.

from which young leaves recovered. At 20° the yellowing was less diffuse and tended to occur as chlorotic spots and blotches. Occasional plants produced necrotic rings. At 16° severe systemic necrosis and very little chlorotic ringing or yellowing occurred. Plants at the low temperatures did not exhibit recovery in the course of the experiment.

TABLE 1.—*Symptoms produced by the watermelon virus on selected greenhouse plants*

Host family and species	Local symptoms	Systemic symptoms
Cucurbitaceae:		
Cucumis sativus L. (cucumber, vars., Maine No. 2, Ohio 31).	Small chlorotic rings followed by general chlorosis of cotyledons; chlorotic lesions on older leaves.	Yellow mottle with distinct chlorotic spots followed by diffuse chlorosis in which lesions lose distinctiveness; severe stunting.
Cucumis melo L. (muskmelon, vars. Golden Delicious, Early Knight).	Chlorotic lesions becoming necrotic and zonate.	Same as above; eventually masked.
Citrullus vulgaris: Schrad. (watermelon, var. Early Kansas).	Irregular to concentrically zonate necrotic lesions.	Severe stunting, distortion of leaves; irregular chlorotic mottle with necrosis.
Cucurbita maxima Duchesne (squash, vars. Early Prolific, Blue Hubbard).	Conspicuous chlorotic lesions followed by extensive necrotic areas.	None for Blue Hubbard and virus not recovered. Occasional single large chlorotic lesions for Early Prolific.
C. pepo L. (pumpkin, vars. Connecticut Field, Sugar).	do	Severe yellow mottle with distortion and stunting. Few plants infected.
Compositae:		
Aster amellus L. (aster)	Necrotic lesions	Yellow-green mottle followed by irregular necrosis.
Calendula officinalis L. (calendula, var. Orange King).	None	None; virus recovered.
Helianthus annuus L. (sunflower, var. Mammoth Russian).	Scattered necrotic lesions	Coarse yellow-green mottle.
Zinnia elegans Jacq. (zinnia, var. Liliput).	Chlorotic lesions	Chlorotic mottle.
Cruciferae:		
Hesperis matronalis L. (dames violet).	None	Slight yellow-green mottle.
Thlaspi arvense L. (pennycress)	do	None; virus recovered.
Capsella bursa-pastoris (L.) Medic. (shepherds-purse).	do	Do.
Brassica napus L. (rape, var. Dwarf Essex).	do	None.
B. oleracea botrytis L. (broccoli, var. Italian Green Sprouting).	do	Do.
B. oleracea gemmifera Zenker (brussels sprouts, var. Long Island Improved).	do	Do.
Leguminosae:		
Pisum sativum L. (pea, vars. Alaska, Pride, Merit, Commando, Prince of Wales).	Irregular necrosis and chlorosis	Severe mottle followed by death of plant.
Melilotus alba Desr. (white sweetclover).	None	None; virus recovered.
M. indica All. (yellow sweetclover).	do	Do.
Phaseolus vulgaris L. (bean. See page 5 for varietal reactions).	Necrotic flecks, streaks or ringspots.	Severe necrosis of growing tip, necrotic flecks and ringspots on leaves and elongate cankers on petioles and stem; severe stunting and mottling on leaves from adventitious buds.
Trifolium pratense L. (red clover).	None	None, virus recovered.
Vigna sinensis Endl. (black cowpea).	Numerous red necrotic lesions	Blotchy mottle with irregular necrosis of stem and petioles; often lethal.
Vicia faba L. (broad bean)	Irregular black necrosis	Black necrosis of leaves and stem lethal.
Solanaceae:		
Capsicum frutescens L. *grossum* Bailey (pepper, var. California Wonder).	None	None.
Lycopersicon esculentum Mill. (tomato, var. Bonny Best).	do	Do.
Nicotiana tabacum L. (tobacco, var. Conn. Havana No. 38).	Scattered etched rings; occasional circular necrotic lesions.	Concentric chlorotic ringing and ringspotting with slight leaf puckering; later replaced by conspicuous diffuse yellowing: in transition from ringing to yellowing marked jagged, lightninglike pattern common; partial masking.
N. glutinosa L.	Very faint chlorotic rings and ringspots.	Faint chlorotic rings and ring spots becoming etchedlike; often no symptoms; complete masking.

TABLE 1.—*Symptoms produced by the watermelon virus on selected greenhouse plants*—Continued

Host family and species	Local symptoms	Systemic symptoms
Solanaceae—Continued		
N. rustica L.	Etchedlike ringing and ringspotting.	Broad, chlorotic rings on young leaves becoming markedly zonate.
Petunia hybrida Vilm. (petunia)	Chlorotic and necrotic lesions	Chlorotic mottle.
Solanum triflorum Nutt.	Scattered necrotic lesions	Chlorotic mottle; stunting and distortion.

PROPERTIES OF THE VIRUS

Maine cucumber plants were used in determining the physical properties. Inoculum in each case was taken from recently infected tobacco plants. The results are given in table 2. The points at which inactivation occurred (dilution, 1 to 100,000; aging in vitro, 5 days at 20° C.; thermal inactivation, 70° for 10 minutes) were similar to those reported for *Cucumber virus* 1 and the tobacco ringspot virus.

TABLE 2.—*Physical properties of the watermelon virus as determined on cucumber*

Type and degree of treatment	Ratio of cucumber plants infected to those inoculated in trial—			
	1	2	3	Total
Dilution:				
0	30/30	20/20	100/100	150/150
1–10	30/30	19/20	100/100	149/150
1–100	29/30	14/20	100/100	143/150
1–1,000	9/30	8/20	9/100	26/150
1–10,000	0/30	2/20	2/100	4/150
1–100,000	0/30	0/20	0/100	0/150
Aging in vitro (hours at 20° C.):				
0	30/30	24/24	50/50	104/104
24	30/30	24/24	50/50	104/104
48	21/30	24/24	43/50	88/104
72	16/30	18/24	31/50	65/104
96	5/30	1/24	0/50	6/104
120	0/30	0/24	0/50	0/104
Thermal inactivation (10 minutes at ° C.):				
Unheated	24/24	24/24	24/24	72/72
45	24/24	24/24	24/24	72/72
50	24/24	24/24	24/24	72/72
55	24/24	24/24	24/24	72/72
60	22/24	24/24	20/24	66/72
65	1/24	7/24	3/24	11/72
70	0/24	0/24	0/24	0/72

Several attempts to transmit the virus from tobacco to cucumber and vice versa by the green peach aphid (*Myzus persicae* L.) were unsuccessful.

IDENTIFICATION OF THE VIRUS

In extensive cross-protection tests, the watermelon virus failed to protect tobacco plants against subsequent infection with *Tobacco virus* 1 and *Cucumber virus* 1, but complete protection was afforded against the tobacco ringspot virus.

The tests of relationship to the tobacco ringspot virus were as follows: Several tobacco plants were inoculated with tobacco ringspot

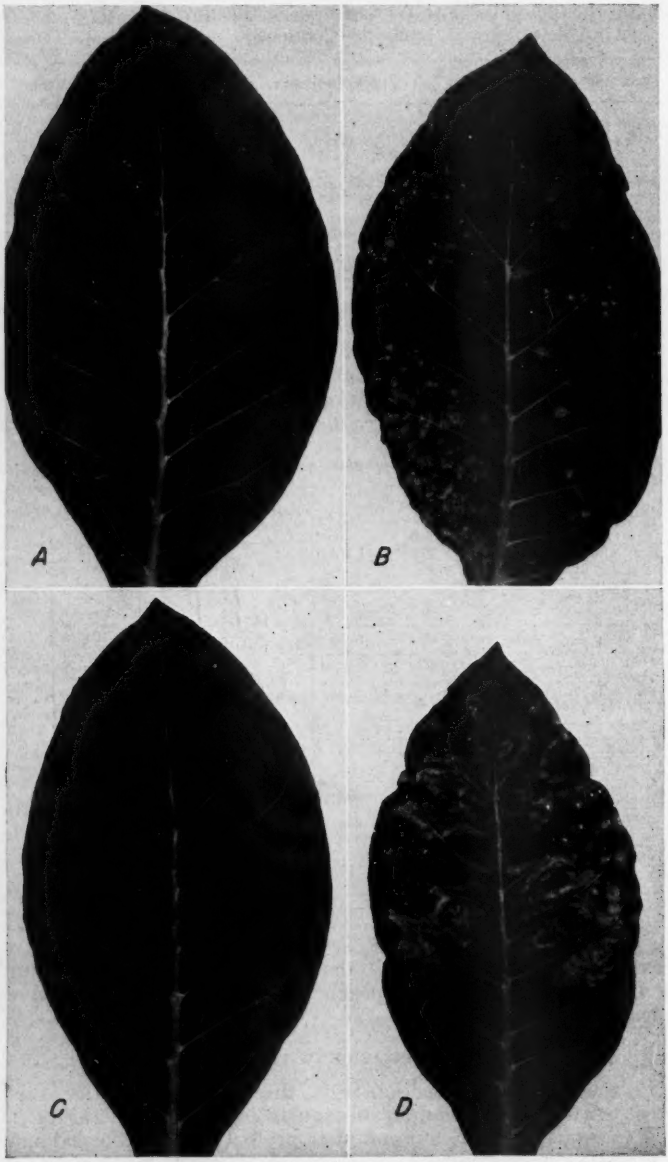

FIGURE 6.—Leaves from plants used in the cross-protection tests with the tobacco ringspot virus. *A*, Inoculated leaf which had recovered from symptoms of tobacco ringspot when reinoculated with the watermelon virus; *B*, leaf of same age which was healthy when inoculated with the watermelon virus; *C*, *D*, leaves from same plants, respectively, but taken above the inoculated leaves shown in *A* and *B*.

virus.[4] After complete recovery occured, the symptomless leaves were reinoculated with the watermelon virus. At the same time healthy plants of the same age were inoculated with the watermelon virus to serve as controls. In all cases (involving six different trials), the watermelon virus produced no symptoms on the ringspot-recovered plants but abundant symptoms on the control plants (fig. 6). This indicated a strain relationship between the watermelon virus and tobacco ringspot virus.

DISCUSSION

Several reports have been made of virus diseases and of viruses affecting watermelon under artificial inoculations. Ainsworth (*1*) reported the infectiousness of *Cucumber viruses* 3 and 4 to watermelon. Porter (*7*) reported that watermelon was highly susceptible to *Cucumber virus* 2. Many strains of *Cucumber virus* 1 produce only local symptoms on watermelon (*3*, *15*), and only rarely have systemic infections of this host been demonstrated. Wellman (*14*) reported systemic infection of watermelon by the celery strain of *Cucumber virus* 1 under greenhouse tests and inferred that mosaic-infected vines observed in nature were of the same cause. Porter and Melhus (*8*) of Iowa, Milbrath (*6*) of California, and Walker (*13*) of Florida reported natural occurrence of watermelon mosaic without identifying the causal virus. The virus producing the watermelon disease described herein was shown to be caused by a yellow strain of the tobacco ringspot virus. It agreed very closely with the latter virus in symptomatology, in host range, properties, and in failure to be transmitted from tobacco to other hosts by the use of the green peach aphid (*Myzus persicae* L.). Furthermore, by host-immunity reactions, its relationship to the tobacco ringspot virus was proved. Wingard (*16*) and others reported the infectiousness of the tobacco ringspot virus to cucurbits in greenhouse tests. Henderson (*4*) reported a natural occurrence of this virus on muskmelon and squash in Virginia, and Valleau (*10*) reported that it caused a mosaic disease of cucumbers in Kentucky.

The regular occurrence of the tobacco ringspot virus in watermelon in Wisconsin is interesting in view of the absence of known insect vectors and its rare occurrence as a disease of proximal tobacco plantings. However, the virus is well-distributed on soybean plantings throughout the North Central States (*2*) and no doubt occurs on many weed hosts as well. Its transmission to watermelon might well be by the same means as its transmission to soybean. Also the possibility of transmission through cucurbit seeds should not be overlooked. Henderson (*5*) and Valleau (*10*) demonstrated that the tobacco ringspot virus is transmitted to a considerable degree in seeds of petunia and tobacco, respectively.

[4] Two different strains of tobacco ringspot were obtained from Dr. R. W. Fulton. One was from infected tobacco in Maryland in 1948. The other came originally from Wisconsin and was shown to immunize against Price's Strain of tobacco ringspot virus.

SUMMARY

A mosaic disease of watermelon and muskmelon in Wisconsin was found to be caused by a yellow strain of the tobacco ringspot virus. The virus was found to be nontransmissible from tobacco to other hosts by the green peach aphid (*Myzus persicae* L.). In physical-property studies, inactivation of the virus occurred at: Dilution, 1 to 100,000; thermal inactivation, 10 minutes at 70° C.; aging in vitro, 5 days at 20°. The watermelon virus effectively protected tobacco plants against subsequent infection with the tobacco ringspot virus. A description of the disease in the field and of symptoms on selected greenhouse hosts is given.

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CYTOLOGY OF REPRODUCTION IN SORGHUM VULGARE[1]

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INTRODUCTION

The embryology of grasses has been studied intensively for over a century, and the literature on this subject is considerable. However, nothing seems to have been published about embryo development in sorghum (*Sorghum vulgare* Pers.) save some observations by Stephens and Quinby (*17*)[3] on fertilization. Anthesis and pollination of grasses including sorghum have been studied by several investigators (*3, 7, 13, 17*), with results that substantially agree. The structure of grass seed has been investigated in relation to physiology (*8*), and that of the mature embryo and seedling in connection with homologies (*2, 12, 14, 15*). Homologies in grass embryos and seedlings have been a matter of controversy for more than a century and today remain one of the most controversial subjects in botany. There is little anatomical evidence that would aid in a purely objective interpretation of the nature of the scutellum, the coleoptile, and the axis interval linking these two organs. The present paper deals with flower and seed development of sorghum, with emphasis on the time factor in fertilization and on embryo development.

MATERIALS AND METHODS

Plants of three sorghum varieties, Folger, Red X, and Collier, were grown in the greenhouses of the Plant Industry Station of the United States Department of Agriculture, at Beltsville, Md., and in field plots at the New Mexico Agricultural Experiment Station, State College, N. Mex. The plants that furnished the material for fertilization studies were rendered male sterile by hot-water treatment of the inflorescences. Viable pollen was then applied, and collections were made at definite intervals. The cytological preparations were made from material killed in Carnoy's solution, embedded in paraffin, and stained with Heidenhain's iron-alum haematoxylin. Pollen germination studies were made on a culture medium containing 1½ percent agar and 40 percent sucrose.

[1] Received for publication July 7, 1948.

[2] Credit is due Percy S. Browne, photographer, Division of Sugar Plant Investigations, for the photomicrographs in plates 1, 3, and 6 to 9, and to Rosemary Schipman for the drawings in figure 1.

[3] Italic numbers in parentheses refer to Literature Cited, p. 672.

MORPHOLOGY OF INFLORESCENCE [4]

The floral organs of sorghum have been described in great detail by Cowgill (*6*), so that only a brief characterization of the inflorescence, or head, is necessary here.

The inflorescence of the varieties of sorghum studied is a rather compact panicle, jointed to the last node of the stalk by the peduncle (fig. 1). The peduncle consists of nodes and internodes. The internodes are often so short that the lateral branches have the appearance of being in whorls. The primary branches divide to form laterals of second and third order.

The joints of the rachis bear paired spikelets. Of each pair of spikelets one is fertile and sessile, the other sterile and pedicellate. In the last joint two sterile spikelets are grouped with a single fertile spikelet.

Each fertile spikelet has two thick, somewhat indurate glumes of about equal length, the lower partially enveloping the upper. The glumes enclose two florets, of which the lower is sterile and the upper perfect. The sterile floret consists of a lemma only. The fertile floret consists of a lemma, a palea, two lodicules, three stamens, and a one-celled ovary with two plumose stigmas. The fertile lemma is thin and hyaline and often bears an awn. The two lodicules lie adjacent to the fertile lemma, at the base of the spikelet.

The sterile spikelet is more elongated than the fertile. It commonly consists of two glumes only, but may have other parts.

MACROSPOROGENESIS

The ovule of sorghum is solitary and is attached with a broad surface to the wall of the carpel. It is at first erect. Subsequently, because of unequal growth in the nucellus, the micropylar end is pushed over until at time of megaspore mother cell formation it is adjacent to the end of the flower (pl. 1, *A*). The ovule continues to rotate until it becomes anatropous, with the micropyle near the hilum. There are two integuments, each two layers thick. The outer integument is much shorter than the inner and never reaches the micropyle (pl. 1, *C*).

The megaspore mother cell arises from a hypodermal cell at the time when the two integuments differentiate. Sometimes the sporogenous tissue consists of two megaspore mother cells occurring side by side within an ovule (pl. 1, *E*). At first each megaspore mother cell is polygonal, is wider at its micropylar end (pl. 1, *A*), and contains a large nucleus and dense cytoplasm. Later it enlarges greatly and becomes oblong (pl. 1, *B*). It abuts externally on a single layer of nucellar tissue, but soon periclinal divisions within this layer increase the width of the nucellar epidermis and cause the megaspore mother cell to be pushed toward the chalazal portion of the nucellus.

The megaspore mother cell undergoes meiosis and becomes a dyad in the normal manner. The second divisions follow quickly, forming a tetrad of megaspores disposed in one longitudinal row (pl. 1, *D*). From the beginning, the chalazal megaspore is larger than the other

[4] The anatomy and morphology of the vegetative organs of sorghum have been discussed by the senior author (*1*).

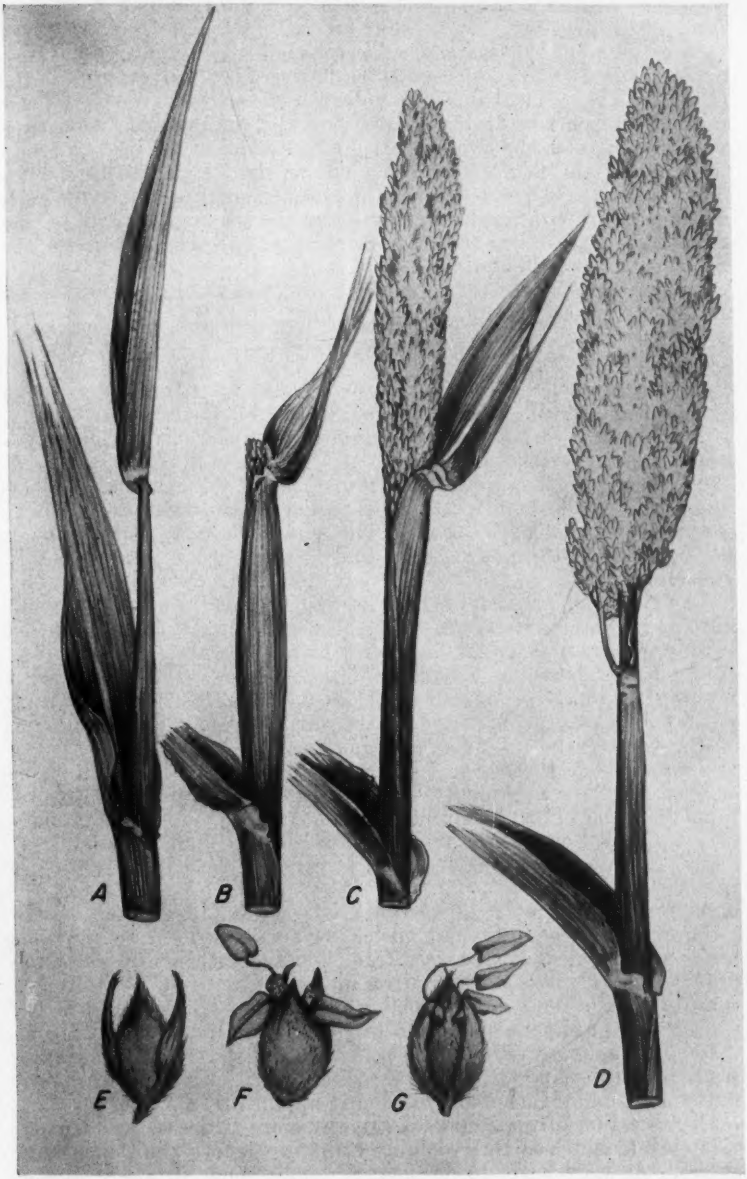


FIGURE 1.—Habit sketches of young inflorescence of sorghum: *A*, Head still enclosed by terminal leaf sheath; *B* and *C*, head emerging; *D*, inflorescence raised by elongation of peduncle; *E*, mature sessile spikelet with two pediceled spikelets; *F*, spikelet at time of anthesis; *G*, spikelet at end of anthesis, with glumes closed.

members of the tetrad. It becomes functional while the others degenerate (pl. 1, *F*).

The functional megaspore enlarges and then divides. Only one nucleus stays at the micropylar end; the other moves toward the chalaza. The chalazal nucleus divides at right angles to the micropylar nucleus and parallel to the long axis of the embryo sac. One large vacuole separates the two nuclear groups. The nuclei of each group then divide, the micropylar in a plane at right angles to that of its first division. The embryo sac now contains eight nuclei. Cell walls are laid down, and soon they transform the macrogametophyte into the typical eight-celled structure consisting of the egg apparatus, the polar nuclei, and the antipodals.

During the nuclear divisions the embryo sac enlarges greatly by absorbing adjacent tissue of the nucellus.

ORGANIZATION OF MATURE EMBRYO SAC

At the time of fertilization the ovule is about 1 mm. long. It is narrow radially (pl. 2, *A*) and broad in the dorsiventral plane (pl. 2, *B*). The embryo sac is oval or somewhat pear-shaped, with the broader end near the micropyle.

The young egg is spherical. As it increases in size it becomes oblong and finally balloon-shaped. It lies between the two synergids and extends somewhat above them. The nucleus is prominent, although not so large as the polar nuclei, and the cytoplasm is prominently vacuolated.

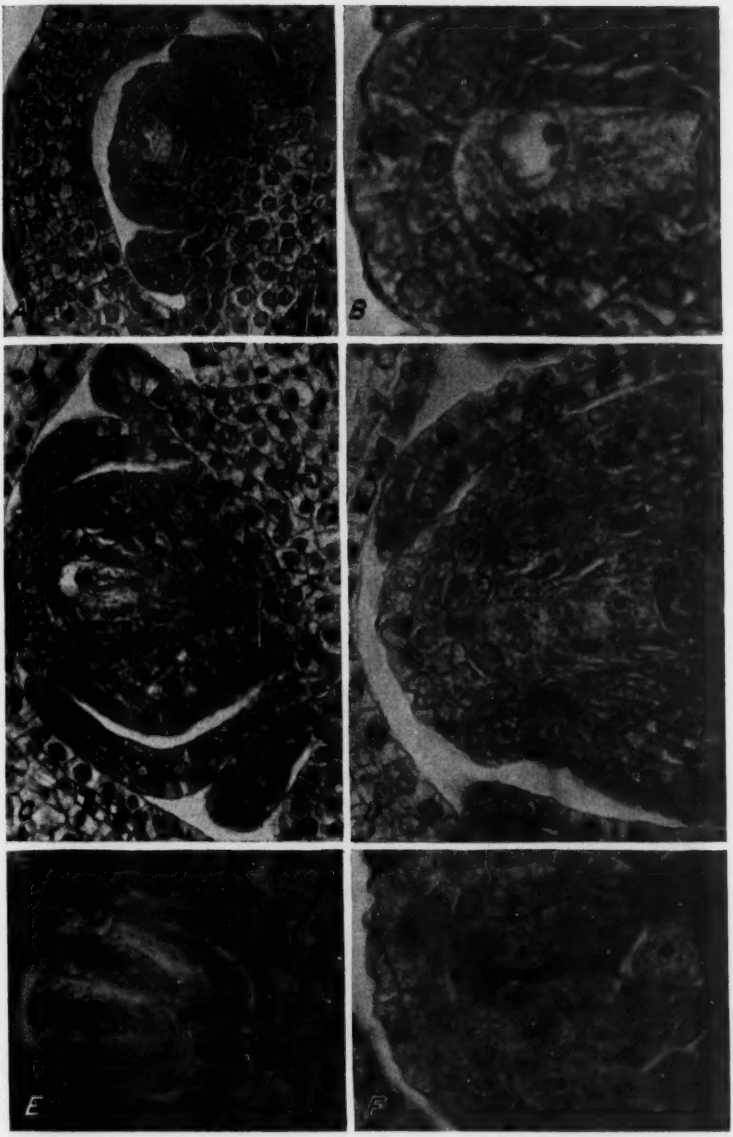
The synergids are sister cells. At first they are pear-shaped, with flattened adjoining sides (pl. 3, *C*). Later the basal part of each cell contracts, and this creates a cleft between them (pl. 3, *D*). The protoplasm is dense and alveolar, and there are no vacuoles; the nuclei are large and have prominent nucleoli (pl. 3, *C*). With the maturing of the embryo sac, the synergids shrink and lose their definite structure.

The polar nuclei (pl. 3, *B*) are found in close contact with the egg apparatus from the time of their differentiation. The contiguous sides of these nuclei are flattened (pl. 3, *E*). Each polar nucleus retains the whole of its membrane until the two fuse to form the primary endosperm nucleus. This fusion occurs either before fertilization or later. The nucleoli of the polars are larger than those of the synergids (pl. 3, *C*).

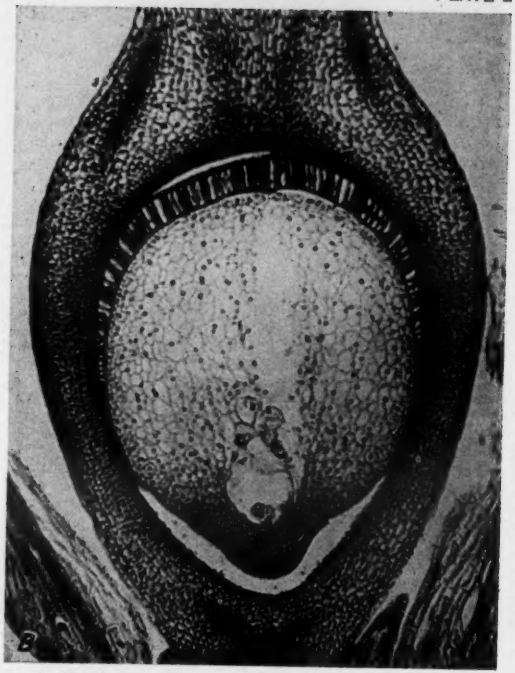
A conspicuous antipodal complex is common to all grasses. In sorghum the three antipodal nuclei begin to divide as soon as the lower polar nucleus is separated from them. Nuclear division is followed by cytokinesis resulting in a network of antipodal tissue that completely fills the chalazal portion of the embryo sac. As the sac enlarges, the antipodal cells also increase in size and become coenocytic, with as many as four nuclei in each cell (pl. 4, *C*). The cytoplasm of the antipodal cells is dense and lacks vacuoles, and their nuclei are smaller than those of the egg apparatus (pl. 3, *A*, *B*).

Degeneration of the synergids sets in before anthesis. The synergids shrink and lose their definite structure before the pollen tube enters. As they begin to degenerate, longitudinal striations appear in their apices, giving rise to the filiform apparatus.

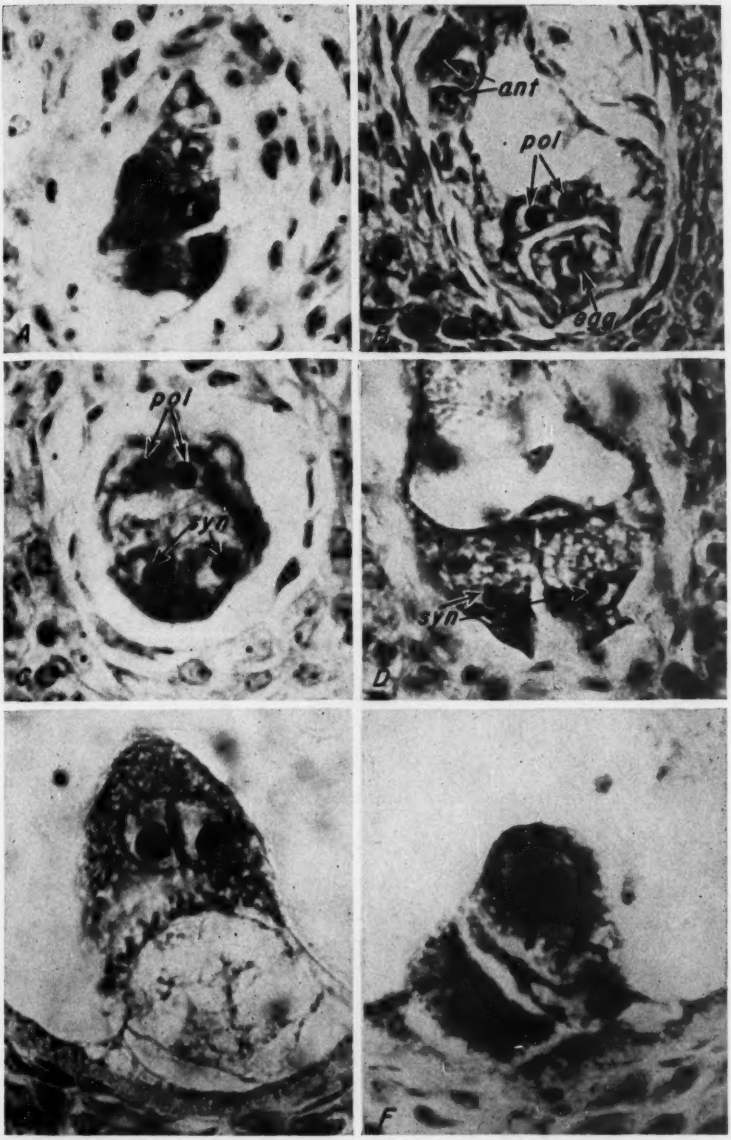
Disorganization of the antipodal complex begins before fertilization. The cytoplasm becomes vacuolated, and the nuclei fuse (pl. 4, *C*). A



Longitudinal sections of ovules of sorghum: *A*, With young megaspore mother cell; × 500. *B*, With older megaspore mother cell; × 1,000. *C*, With megaspore mother cell at late telophase; × 500. *D*, With linear tetrad of megaspores; × 700. *E*, With two megaspores at first metaphase, lying parallel to each other; × 800. *F*, With chalazal megaspore functional and others degenerated; × 800.

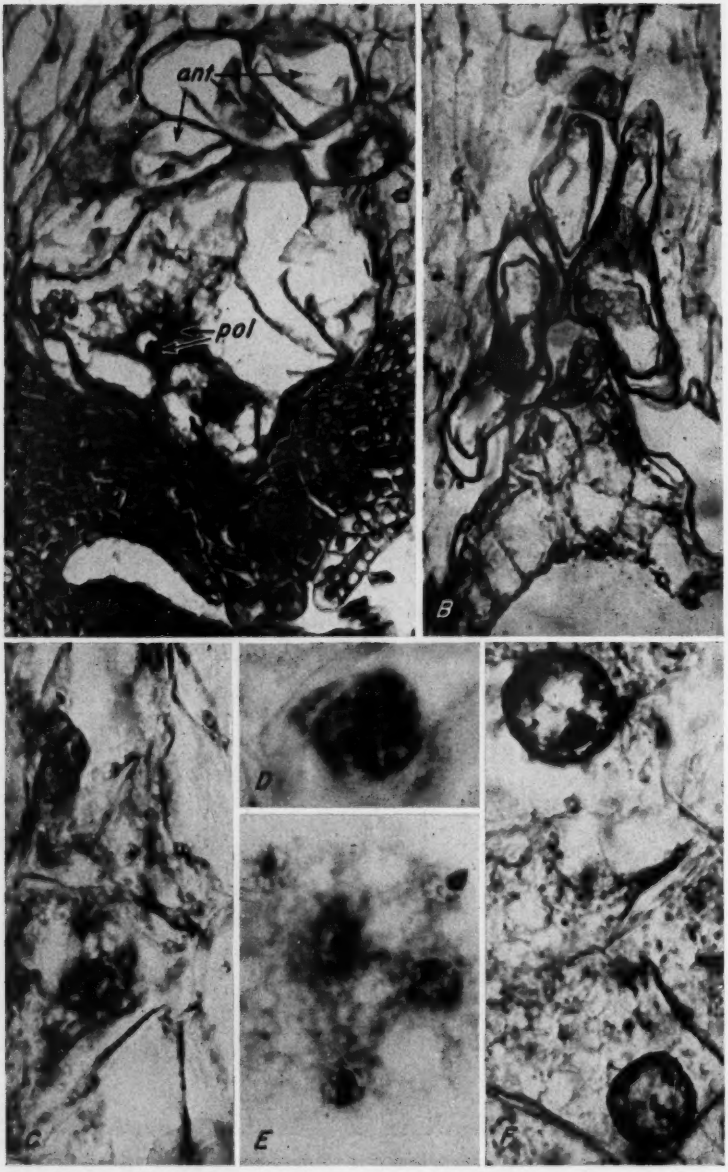


Ovule and embryo sac at time of fertilization: *A*, Median sagittal section; × 65.
B, Median face longitudinal section; × 85.



Longitudinal sections of embryo sacs: *A*, Young antipodal complex; × 850. *B*, Young antipodal complex, polar nuclei, and egg; × 700. *C*, Synergids and polar nuclei; × 850. *D*, Synergids at time of fertilization; × 850. *E*, Polar nuclei at time of fertilization: × 850. *F*, Fertilized egg; × 850. *ant*, Antipodal nuclei; *pol*, polar nuclei; *syn*, synergids.

B, Median face longitudinal section; × 85.



Longitudinal sections of embryo sacs at time of fertilization: *A*, Sac with greatly enlarged antipodal cells; × 350. *B*, Large antipodal cells spearheading endosperm tissue into disintegrating nucellus; × 350. *C*, Antipodal nuclei in state of fusion and disintegration; × 850. *D*, Degenerating antipodal nucleus; × 1,700. *E*, Nuclei of young endosperm; × 850. *F*, Nuclei of old endosperm; × 850. *ant*, Antipodal cells; *pol*, polar nuclei.

typical fusion nucleus is shown in plate 4, *D*, together with numerous chromatin granules.

ANTHESIS

The order of anthesis of sorghum has been recorded in detail by several investigators (*3*, *7*, *13*, *17*). According to their observations, the reproductive phase is indicated by a characteristic swelling of the apex of the stalk. The swollen area is enclosed by the terminal leaf sheath (fig. 1, *A*), whose blade, the flag, is usually small and inconspicuous. Emergence of the flag is followed by the ushering out of the boot. Next the inflorescence proper makes its appearance (fig. 1, *B*). When the head is completely clear the peduncle further elongates (fig. 1, *D*), elevating the head to a height characteristic of the variety. According to Ayyangar and Rao (*3*), about 6 days are required for the emergence of the flag, at least about 9 days for the ushering out of the boot, 5 or more days for the complete clearance of the head, and 5 more days for the elongation of the peduncle.

Anthesis in the inflorescence is basipetal. The length of time required for all flowers of a panicle to open averages 7 days, according to Graham (*7*), and maximum blooming takes place on the third or fourth day. According to detailed observation by Stephens and Quinby (*17*), usually the terminal or the second flower of the uppermost panicle branch opens first, and, in general, the flowers in any horizontal plane within the panicle open at the same time.

Blooming takes place chiefly in early morning; the largest anthesis occurs as early as 2 a. m. The opening of the individual flower is rapid, owing to pressure produced by the simultaneous swelling of the two lodicules. The bracts move apart until they form an obtuse angle, the whole movement being completed in about 10 minutes. As the glumes fan out, the stigmas and anthers emerge. The anthers become pendent almost immediately. Usually the stigmas become visible (fig. 2) before the filaments of the anthers elongate. The period during which the flowers remain open ranges, according to different observers (*3*, *6*, *16*), from 30 to 90 minutes.

MICROSPOROGENESIS

The anthers of sorghum appear first as a mass of homogeneous cells, which soon becomes four-lobed in cross section (pl. 5, *A*). The primary archesporium in each of the 4 lobes forms a plate 15 to 20 cells long and 2 cells broad, enclosed on the outside by an epidermis and 3 wall layers (pl. 5, *B*). The wall layer next to the epidermis forms the fibrous endothecium of the mature anther. The cells of the innermost layer differentiate into the tapetum (pl. 5, *C*, *D*). The layer between the tapetum and the endothecium disorganizes early and becomes completely resorbed.

The tapetum attains its maximum development at the first reduction division in the pollen mother cells. The cells are uninucleate but become binucleate with the advent of meiosis. As the pollen tetrads are being formed, the nuclei and cytoplasm of the tapetum take on a dark stain. Signs of degeneration of the tapetum appear at this stage, and by the time the microspores separate only vestiges of this layer remain (pl. 5, *E*). The epidermal cells of the anther enlarge greatly and become more massive than any of the cells of the wall layers (pl.

5, *D*, *E*) except at the two ends of the anther, where the endothecium is conspicuous.

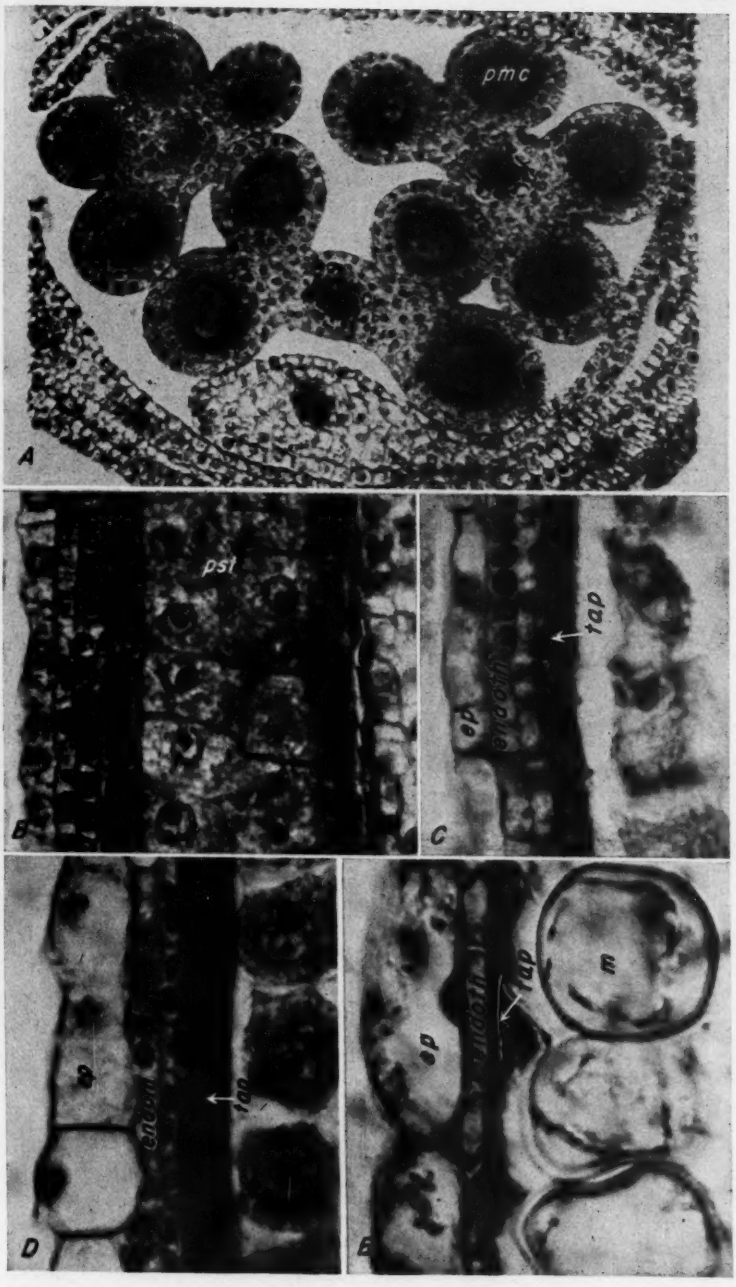
While the anther wall progressively differentiates, the primary sporogenous tissue divides to form pollen mother cells and each of these undergoes meiosis in the normal manner (pl. 6, *A–E*). No wall is formed at the close of the first division. The second division produces two spindles. Either the spindles are nearly at right angles to each other (pl. 6, *C*) and the microspores are in tetrahedral arrangement or the spindles are parallel to each other and the microspores are within the same plane (pl. 6, *E*). Cytokinesis takes place through simultaneous furrowing from four sides. Soon after liberation from the tetrad, the pollen grains round off and increase considerably in size (pl. 5, *E*). They develop the usual exine and intine. The former is smooth and is perforated by one germ pore (pl. 6, *G*).

The pollen grains are usually trinucleate by the time the exine is fully developed (pl. 6, *F*, *G*). The tube nucleus is large and of spherical or irregular shape. It stains rather weakly if at all. The sperms are small and usually slightly elongate. While most pollen grains have only two sperms, grains with supernumerary sperms are not uncommon (pl. 6, *H*). The

FIGURE 2.—Branch of inflorescence at anthesis.

EXPLANATORY LEGEND FOR PLATE 5

Microsporogenesis: *A*, Cross section of young flower with anthers at time of pollen mother cell formation; × 275. *B*, Longitudinal section of young anther with primary sporogenous tissue and all wall layers differentiated; × 850. *C*, Pollen mother cell at early prophase, tapetal cells enlarged and uninucleate; × 850. *D*, Pollen mother cell at late prophase, binucleate tapetal cells, and cells of anther epidermis; × 1,000. *E*, Anther with pollen grains mature, tapetal cells degenerated, and third wall layer between endothecium and tapetum completely resorbed; × 1,000. *endoth*, Endothecium; *ep*, epidermis; *m*, microspore; *pmc*, pollen mother cells; *pst*, primary sporogenous tissue; *tap*, tapetum.


pmc
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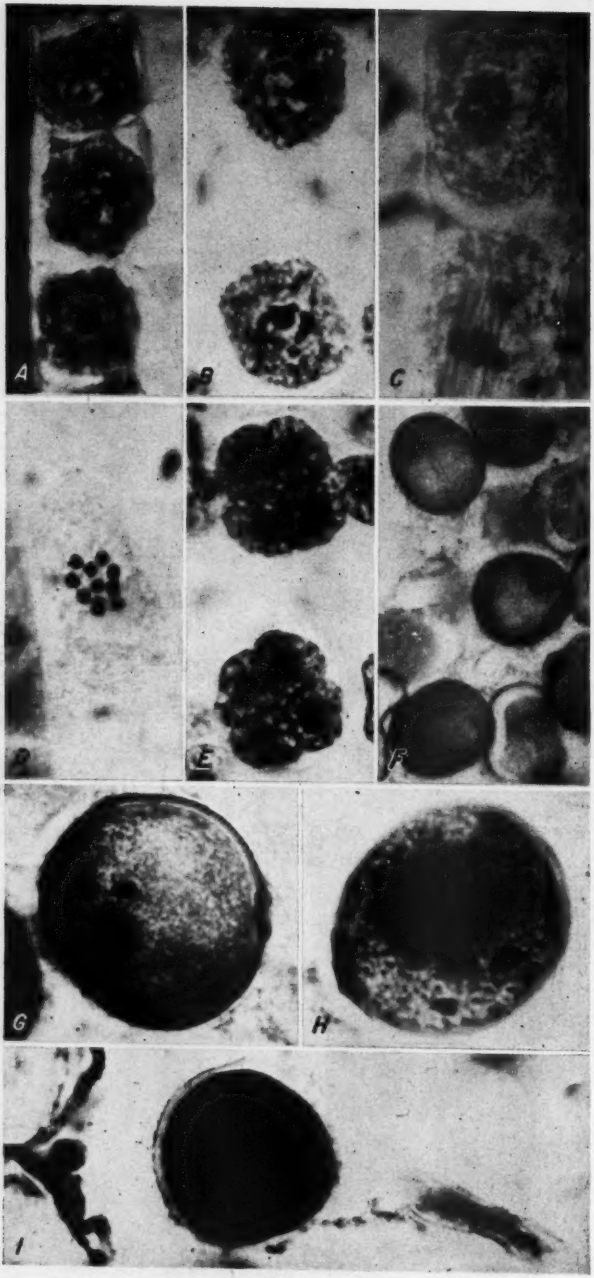
A
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haploid chromosome complement as seen at the first meiotic metaphase in pollen mother cells is 10 (pl. 6, *C*, *D*), according to counts made in this investigation and in earlier ones (*9*, *10*, *11*).

FERTILIZATION

Germination tests with sorghum pollen to determine its longevity under different environmental conditions have been made by several investigators.

Stephens and Quinby (*17*) found that pollen lost its vitality within 5 hours, but Patel and Patel (*13*) reported germination after as long as 30 hours. Ayyangar and Rao (*4*) stored packets of pollen in beakers within moist chambers and in open tubes within a refrigerator at 10° C. In the first experiment they obtained about 28 percent germination after 48 hours; reporting on the second, they stated (*4*, *pp. 1311 and 1313*) that "life lingered till even the sixth day" and that "the pollen is capable of fertilising up to the third day though in a very weak measure." The distinctly beneficial effect of low temperatures in this experiment explains why the cold morning hours tend to be most favorable for pollen germination under field conditions. These investigators reported that only 31.2 percent of the pollen gathered as early as 2 a. m. had germinated, but more than 50 percent of that collected between 5 and 8 a. m. had done so. They noted that darkness had an inhibiting effect on pollen germination. This explains the fact that under field conditions germination is deferred to daybreak.

Ayyangar and Rao (*4*) studied pollen germination and pollen-tube growth on artificial media. Sowing pollen on 41-percent sucrose agar, they found that germination usually started within 15 to 30 minutes and that growth was completed in 3 to 4 hours. Rate of growth lacked uniformity. In general, there was a preliminary period of growth at a moderate rate, then one of rapid elongation, and then a slowing down, the decline in rate being more gradual than the early acceleration. The pollen tubes grown on artificial media never developed to such length as would be required to reach the ovary.

Results of pollen germination tests made at State College, N. Mex., during the summer of 1947 were not conclusive. The nutrient medium recommended by Ayyangar and Rao (*4*) proved unsuitable; pollen tubes grown on it burst before they attained appreciable length. Variations in the osmotic concentration of the medium, also, made it unsatisfactory.

Germination of pollen grains within unopened anthers was observed in material collected at State College, N. Mex., during the summer of

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EXPLANATORY LEGEND FOR PLATE 6

Microsporogenesis: *A*, Late prophase; × 1,000. *B*, Diakinesis; × 1,000. *C* and *D*, First meiotic metaphase (10 chromosomes); × 1,700. *E*, Pollen tetrads; × 1,000. *F*, Pollen grains with three nuclei each; × 400. *G*, Mature pollen grain having three nuclei and single germ pore; × 1,000. *H*, Pollen grain with supernumerary sperms; × 1,000. *I*, Germinating pollen grain within unopened anther; × 1,000. *sp*, Sperms.

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1947. Because of crowding, the pollen tubes were twisted and coiled. The sperms and the tube nuclei had migrated into the pollen tubes (pl. 6, *I*), usually to some distance from the tip. The tips of some of the pollen tubes were partly wedged between the cells of the endothecium, but complete penetration of the anther wall has not been seen.

Pollen falling upon a receptive stigma germinates immediately if conditions are favorable. The stigma is receptive sometimes before blooming and always for a long time after it. If pollination has not taken place, the extruded stigmas remain feathery and fresh looking for several days. Stephens and Quinby (*17*) reported that flowers pollinated 8 days after anthesis produced seed and that in cool autumn weather successful pollinations were made even 16 days after blooming.

The pollen tube grows through the stigmatic papillae into a lateral branch of the stigma and thence downward until it reaches the conducting tissue of the style. Upon entering the ovary cavity, the tube grows between the outer integument and the ovary wall and enters the embryo sac by pressing apart the cells of the nucellus (pl. 7,*B*). At this stage the cells lining the micropyle have grown far beyond the prevailing size of the nucellar cells; the outer ones are especially large and protrude (pl. 7, *A*, *B*).

Many pollen tubes grow down the stylar canal into the cavity of the ovary; but only one or two, apparently, penetrate the micropyle and discharge their sperms.

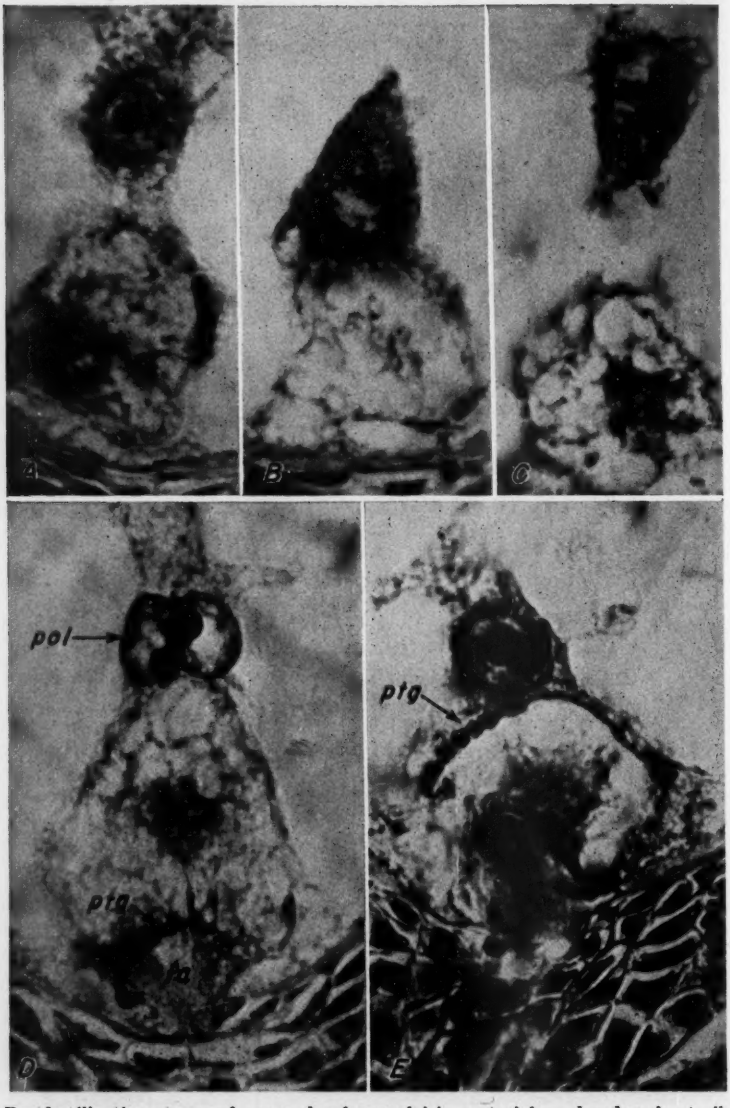
Sperms were observed frequently in the vicinity of the egg and even inside the egg membrane, but actual fusion between sperm and egg nucleus has not been seen. A characteristic accompaniment of fertilization is the appearance of numerous black granules, arranged in a crescent, between egg and polars (pls. 7, *A*, *C*, and 8, *A*, *E*). These granules closely resemble the male nuclei in size and staining reaction; usually they cannot be distinguished from them.

Simultaneously with fertilization of the egg nucleus, that of one polar nucleus or of fused polar nuclei occurs. Even when a pair of polar nuclei are in close contact with each other, fertilization of one may take place without dissolution of the membranes separating the two (pl. 8, *C*). Apparently only one of the polar nuclei is fertilized if the two are in process of fusing. If these nuclei have not fused before fertilization, they do so immediately thereafter. Fertilization is indicated by the presence of an extra nucleolus, which is not so large as the two others but may be almost as large (pls. 7, *E*, *F*, and 8, *C*).

Stephens and Quinby (*17*) studied the time interval between pollination and fertilization on Spur feterita flowers. They found no divisions of egg cell and endosperm nuclei in ovules examined 6 hours or less after pollination; but in ovules examined 12 hours after pollination they found that fertilization had taken place and, in some instances, the endosperm nucleus had divided several times. They concluded that fertilization in sorghum commonly takes place within 8 to 10 hours after pollination, but that the volume of material they examined was too small to exclude the possibility that fertilization can occur within less than 6 hours or after more than 12 hours.

Controlled-pollination experiments with sorghum at Beltsville, Md., showed that pollen tubes and occasionally pollen-tube granules (pl. 8,

A, Section of micropylar end of ovule showing micropyle, crescent of pollen-tube granules, and one polar nucleus; × 500. *B*, Pollen tube in micropyle; × 630. *C*, Fertilized egg, within crescent of pollen-tube granules, and polar nucleus; × 850. *D*, Fertilized egg with two nucleoli; × 700. *E*, Fertilized egg with two closely united polar nuclei in lateral position; × 500. *F*, Fertilized primary-endosperm nucleus with three nucleoli; × 850. *G*, Fertilized egg with two nucleoli; × 850. *H*, Dividing zygote at metaphase; × 800. *I*, Two-celled embryo; × 850. *microp*, Micropyle; *pol*, polar nucleus; *ptg*, pollen tube granules.



Postfertilization stages of egg and polar nuclei in material rendered male sterile through hot-water treatment of the inflorescence: *A*, Fertilized egg and polar nucleus with partial crescent of pollen-tube granules. *B*, Polar nuclei with chromatin in spireme. *C*, Fertilized polar nuclei. One cell has two nucleoli, the other has one. *D*, Fertilized egg and polar nuclei. Synergids, at base of egg, have degenerated into striated filiform apparatus. *E*, Fertilized egg and polar-fusion nucleus separated by crescent of pollen-tube granules. All × 850. *fa*, Filiform apparatus; *pol*, polar nucleus; *ptg*, pollen-tube granules.

D) are present in the micropyle as early as 60 minutes after pollination; material collected 100 to 120 minutes after pollen was applied yielded evidence of fertilization in practically every flower. Under field conditions at State College, N. Mex., evidence of fertilization of sorghum was noticed in material collected at 7 a. m., about 2 hours after sunrise, and 90 percent of the material collected at 8 a. m. was fertilized. Thus, the time factor for fertilization of sorghum is approximately 2 hours, a period much shorter than that given by Stephens and Quinby (*17*). The discrepancy may be explained by the fact that those investigators disregarded the rest period between fertilization and division of the zygote.

POSTFERTILIZATION DEVELOPMENT

The newly fertilized egg contains two nucleoli, one larger than the other (pls. 7, *D*, *G*, and 8, *D*). Fusion of the two nucleoli occurs shortly after fertilization, for only one nucleolus is present when the primary-endosperm nucleus divides.

The zygote and the primary-endosperm nucleus do not divide immediately after fertilization; they undergo a rest period, which is short for the endosperm nucleus but of considerable duration for the zygote. During this rest period general growth processes in the embryo sac and elsewhere in the ovule continue.

The filiform apparatus resulting from degeneration of the synergids extends fanlike above the micropyle even after fertilization (pl. 8, *D*).

The life of the antipodal cells does not terminate with fertilization. While their nuclei degenerate, the antipodals continue to enlarge (pl. 4, *A*, *B*) and their walls thicken appreciably. They retain their position near the chalazal end. As endosperm development progresses, they spearhead the new tissue into the disintegrating nucellus (pl. 4, *B*). Remnants of the antipodal cells may be recognized in ovules with 5-day-old embryos. They completely disappear before the endosperm reaches the distal end of the inner integument.

The primary-endosperm nucleus is usually situated above the egg (pl. 8, *A*, *B*), but often assumes a lateral position just before it divides (pl. 7, *E*). The daughter nuclei resulting from its first division separate immediately; one retains its position, and the other moves to the opposite side of the egg. A second and a third division follow, so that when the rest period of the egg comes to an end four or more endosperm nuclei are present in the embryo sac (pl. 4, *E*).

Two-celled embryos were found abundantly in material collected at 11 a. m. from field-grown plants at State College, N. Mex., and signs of fertilization were not observed before 7 a. m. It appears that the length of the rest period is about 4 hours, or double the time it takes for the pollen tube to traverse the length of the stigma and discharge the sperms into the embryo sac.

THE CARYOPSIS

DEVELOPMENT OF EMBRYO

The fertilized egg divides transversely into two cells (pl. 7, *H*, *I*). The basal cell then divides again, also transversely. Thus wall formation in early embryogeny is in basipetal succession for sorghum as for other grasses (*5*, *16*). The third division is in the apical cell

and is vertical. The fourth occurs in the basal cell and cuts off the suspensor.

The suspensor initial divides several times, transversely. The newly formed cells enlarge greatly (pl. 9, *A*, *B*) and push the embryo away from the micropyle (pl. 9, *C*, *D*). The suspensor is visible as a distinct organ up to the seventh day (pl. 10, *A*), but finally it is crushed and absorbed by the cells of the endosperm.

Data on growth and differentiation in the embryo are given in table 1. Growth is at first symmetrical. The young embryo is filiform, then it is club-shaped—pointed at the micropylar and blunt at the distal end. By the sixth day it has become roughly lozenge-shaped; it is thickest at the middle and its lower end is attenuated and is continuous with the suspensor (pl. 10, *A*).

The first visible sign of organ differentiation is development of an indentation below the apex of the posterior side of the embryo. The portion above the notch develops into the upper part of the scutellum. Below the notch a ring of tissue bulges upward, enclosing the growing point of the stem apex (pl. 11, *A*). The edges of the concentric bulge ultimately unite, with the result that the coleoptile is formed. Within the coleoptile there develop in rapid succession the initials of several foliage leaves.

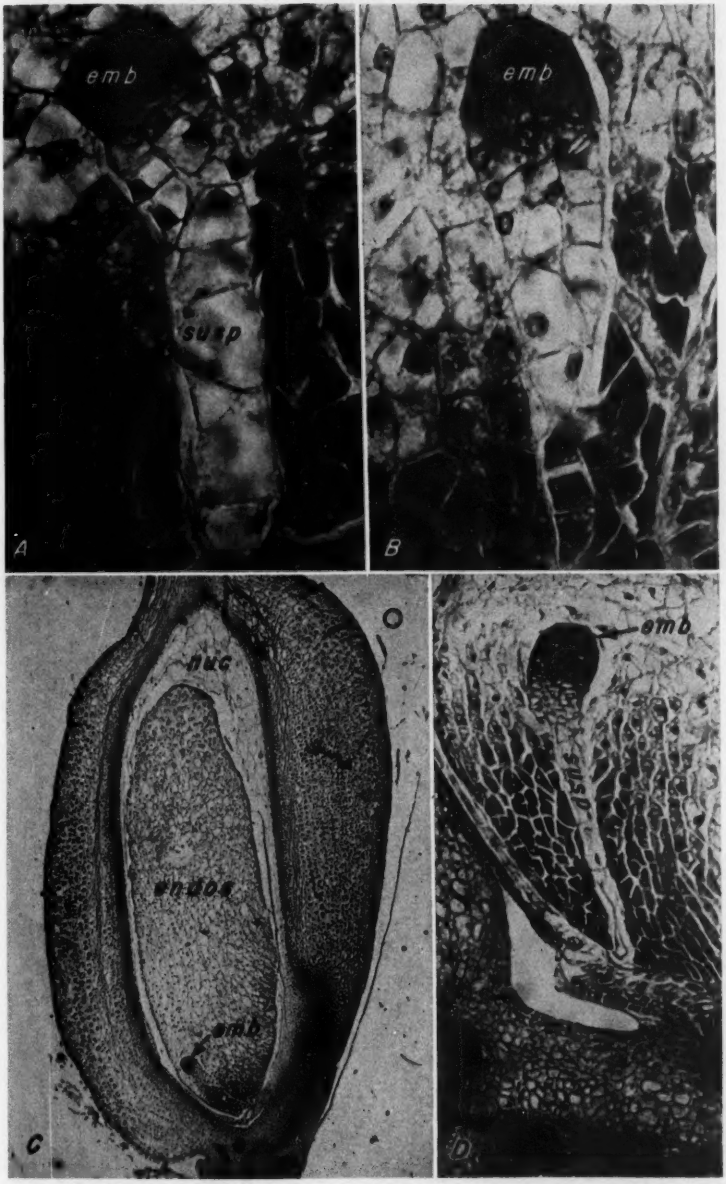
During the period of expansion and differentiation the scutellum is rapidly enlarging, mostly in its upper part (pl. 10, *B*). The lower part, once it begins to grow, develops rapidly (pl. 10, *C*). It reaches the micropylar end of the sac before the twelfth day (pl. 10, *D*). As the scutellum enlarges it expands laterally into two wings, which fold to form a jacket enclosing the embryo. The cells of the scutellum in contact with the endosperm form a weakly differentiated epithelium, which becomes conspicuous as soon as the seed begins to germinate.

TABLE 1.—*Representative measurements of ovules, endosperms, and embryos of Sorghum vulgare and ages of embryo at which internal differentiation took place*

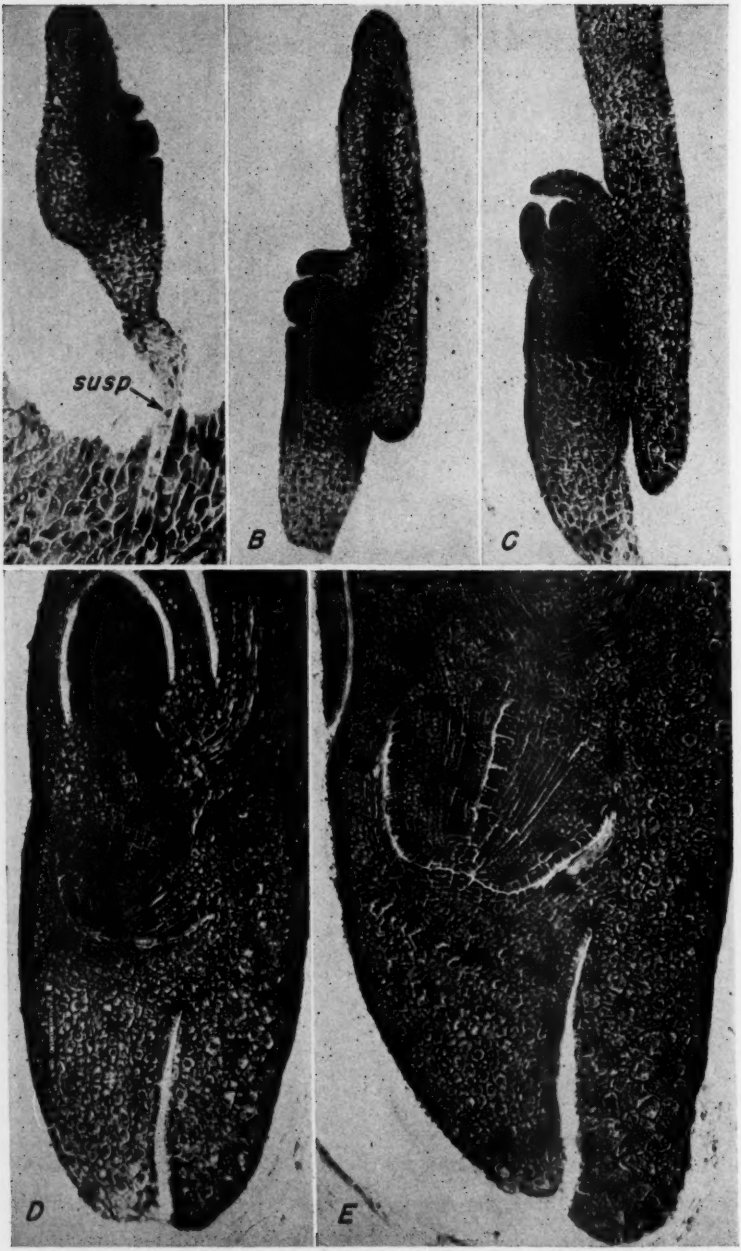
Age of embryo	Ovule length	Endosperm length [1]	Embryo			Suspensor length [2]	Internal differentiation of embryo
			Width	Length	Form [2]		
	μ	μ	μ	μ		μ	
3 hours	900	0	23	36	2-celled	0	
1 day	1,230	410	23	38	Filiform	60	
3 days	2,040	1,212	38	70	Ligulate	245	
4 days	2,380	1,640	69	92	Club-shaped	280	
5 days	2,580	2,340	85	105	do	335	
6 days	2,600	2,350	85	164	do	335	Starch in endosperm.
7 days	2,700	2,400	140	420			Plumule.
8 days	3,110		192	828			Procambium.
9 days	3,120		245	980			Aleurone layer.
10 days	3,200		315	1,150			Root cap.
11 days	3,200		315	1,400			Metaxylem vessels.
13 days	3,940		450	1,800			
19 days	3,940		656	2,620			

[1] After 7 days nucellus had been completely absorbed by endosperm.
[2] After 6 days organs were being differentiated within embryo.
[3] After 6 days suspensors were not distinguishable.

With the appearance of the first-foliage-leaf initial, active cell division begins in the meristematic zone in the lower part of the embryo, with the result that the primary root is formed (pl. 10, *D*). A progressively widening cleft splits the outer parenchyma tissue

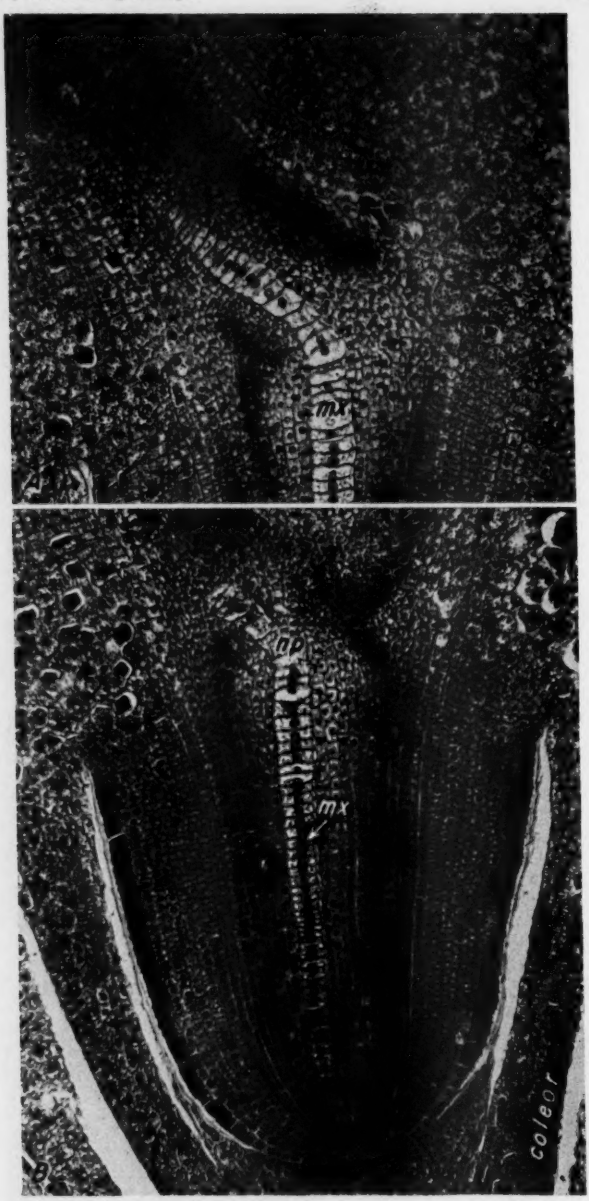


Early embryogeny: *A*, Young embryo with large suspensor; × 525. *B*, Four-tiered embryo with upper part of suspensor showing; × 525. *C*, Ovule with 5-day-old embryo, in which endosperm has replaced almost all nucellar tissue; × 30. *D*, Four-day-old embryo with long suspensor; × 155. *emb*, Embryo; *endos*, endosperm; *nuc*, nucellus; *susp*, suspensor.



Embryo development: *A*, 6-day-old embryo with long suspensor; *B*, 7-day-old embryo; *C*, 8-day-old embryo; *D*, 11-day-old embryo; *E*, basal part of 13-day-old embryo. All × 135. *susp*, Suspensor.

Median sagittal sections of (*A*) mature embryo and (*B*) lower part of mature embryo. *A*, × 65. *B*, × 75. *col*, Coleoptile; *coleor*, coleorhiza; *ff*, first foliage leaf; *gp*, growing point; *np*, nodal plate; *pr*, primary root; *rc*, root cap; *sc*, scutellum; *sct*, scutellum trace.



A, Longitudinal section through nodal plate and adjacent tissues of embryo, showing continuity of metaxylem vessel; × 150. *B*, Longitudinal section through root zone and nodal plate of embryo; × 150. *coleor*, Coleorhiza; *mx*, metaxylem vessel; *np*, nodal plate.

away from the root proper (pl. 10, *E*). As the rootlet increases in length the cleft extends downward, so that at maturity the lower part of the tissue becomes a sheath (pl. 11, *B*), the coleorhiza, enclosing the root proper. Between the tip of the root and the root sheath is a triangular group of cells, the root cap, which develops through the activity of a calyptrogen located in the upper part of the root cap, at the base of the rootlet.

During the first 12 days growth and tissue differentiation proceed rapidly, especially in the root region. Strands of procambium are definitely evident in the scutellum as early as the eighth day, and the large metaxylem initials of the axis appear the eleventh day. The embryo is completely formed by the twelfth day. It rapidly increases in bulk until the seed is mature.

DEVELOPMENT OF ENDOSPERM

The four or more free nuclei formed within the embryo sac by division of the primary-endosperm nucleus take a peripheral position, leaving a large central vacuole (pl. 4, *E*). Wall formation takes place, proceeding toward the center until the whole sac is completely filled. As the embryo grows, the endosperm forms rapidly (pl. 9, *C*) until it has completely displaced the nucellar tissue. At the base of the ovule the endosperm cells are small, elongated, and angular. The nuclei of the mature endosperm cells (pl. 4, *F*) are very large, often measuring 15 μ in diameter.

Deposition of starch begins on the sixth day in the cells at the end of the caryopsis farthest from the embryo and proceeds downward and outward until all cells are filled except those of the surface layers. The outermost cells, forming the aleurone layer, are roughly rectangular and much smaller than the other cells of the endosperm. This layer becomes distinct when the embryo is about half grown, approximately on the ninth day after anthesis.

STRUCTURE OF MATURE EMBRYO

The mature embryo, which lies in the basal portion of the caryopsis, consists of a primary root, a short axis, the terminal plumule, and the scutellum. The plumule is enclosed within the coleoptile, and the primary root is sheathed by the coleorhiza.

The mature embryo has a complete procambial system but no mature vascular elements. The rootlet is polyarch, with nine protoxylem-group initials arranged in a concentric ring. Near the center are two metaxylem vessels of very large lumen. They extend upward to the base of the coleoptile node, there bend obliquely outward, and soon terminate. These vessels can be traced from the tip of the root through the first transaxis plate, at which they are slightly deflected (pl. 12, *A*, *B*), and on up the stem.

A cross section just above the upper level of the coleorhiza cuts through the first nodal or transaxis plate (pl. 13, *A*, *C*). This region is characterized by transversely or obliquely running procambial bundles. Above the nodal plate the various strands are arranged in two groups of unequal size, which are separated from each other by two metaxylem vessels. The larger of the two groups is deflected obliquely outward toward the scutellum, taking on the shape of a

horseshoe in its outward passage (pl. 13, *B*). The terminal part of the horseshoe-shaped group extends out into the scutellum (pl. 13, *D*), and the two wings of the horseshoe ascend to the coleoptile node, where they supply the traces for the coleoptile (pl. 13, *F*) and take part in the vascularization of the leaves of the plumule. The smaller of the two groups formed above the nodal plate, designated by Sargant and Arber (*15*) the median trace (pl. 13, *D*), ascends unchanged to the base of the coleoptile node and then breaks up into several strands, which extend obliquely outward and supply the principal bundle and some of the lateral bundles of the first foliage leaf (pl. 13, *E*).

STRUCTURE OF SEED COAT

The seed coat of sorghum consists of fused pericarp and testa (pl. 14, *A*), as described and illustrated by Winton (*19*) and Swanson (*18*). The pericarp is essentially the ovary wall. At maturity it has about the same number of cell rows as at time of fertilization; changes during embryo development are restricted to cell growth and cell-wall thickening.

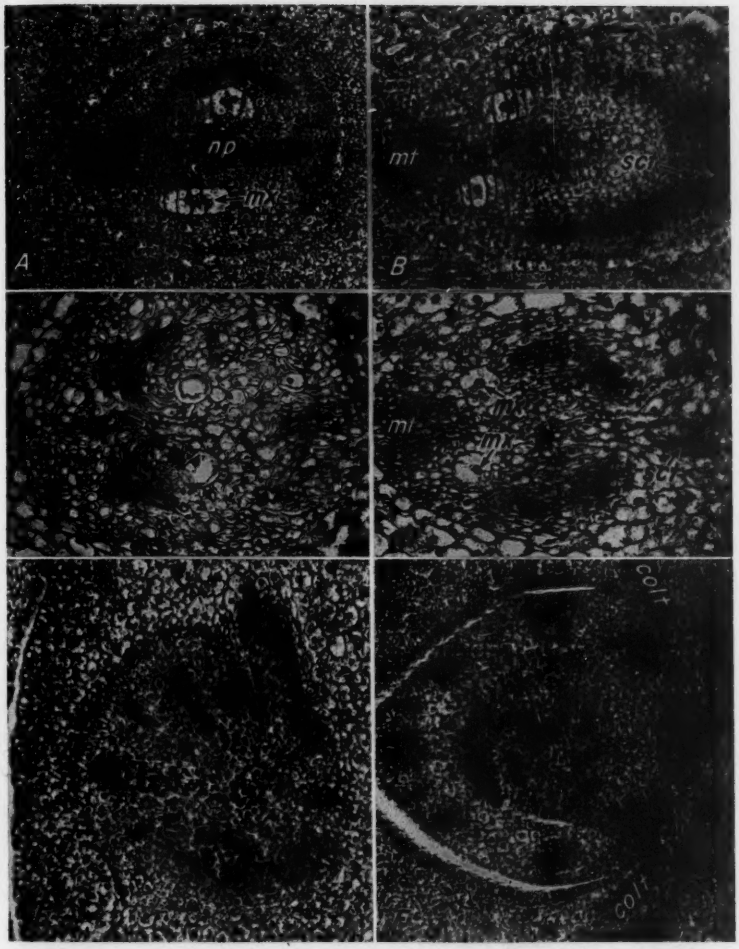
In the mature seed coat the outer portion of the epidermis of the pericarp is made up of a layer of small, thick-walled cells capped by a heavy cuticle (pl. 14, *B*). The cells of the next two or three layers are also thick-walled but much larger. Next comes the broad inner zone, or mesocarp, composed of large, thin-walled elongated cells containing starch. This layer varies in thickness, being widest in the region opposite the embryo (pl. 14, *A*). According to Winton (*19*), it varies in thickness to a marked degree among different varieties of sorghum. The inner layer of the epidermis is composed of long tubular cells extending lengthwise of the seed. In cross section these cells appear circular or roughly elliptical (pl. 14, *B*). Just inside this layer are the cross cells—narrow, elongated elements forming a narrow jacket one or several layers thick.

The testa (pl. 14, *B, C*) is ontogenetically the inner layer of the inner integument and is conspicuous because of its yellow or brown color. It is continuous except at the hilum. Toward the distal end the cells of the testa are greatly elongated. The palisadelike form these cells have at time of fertilization (pl. 2, *A, B*) is more pronounced at later stages. When the embryo is approaching maturity these cells have enlarged and their inner end walls have thickened greatly, but their radial walls remain relatively thin. The cells of the testa are thinner and lighter colored over the embryo than in any other region.

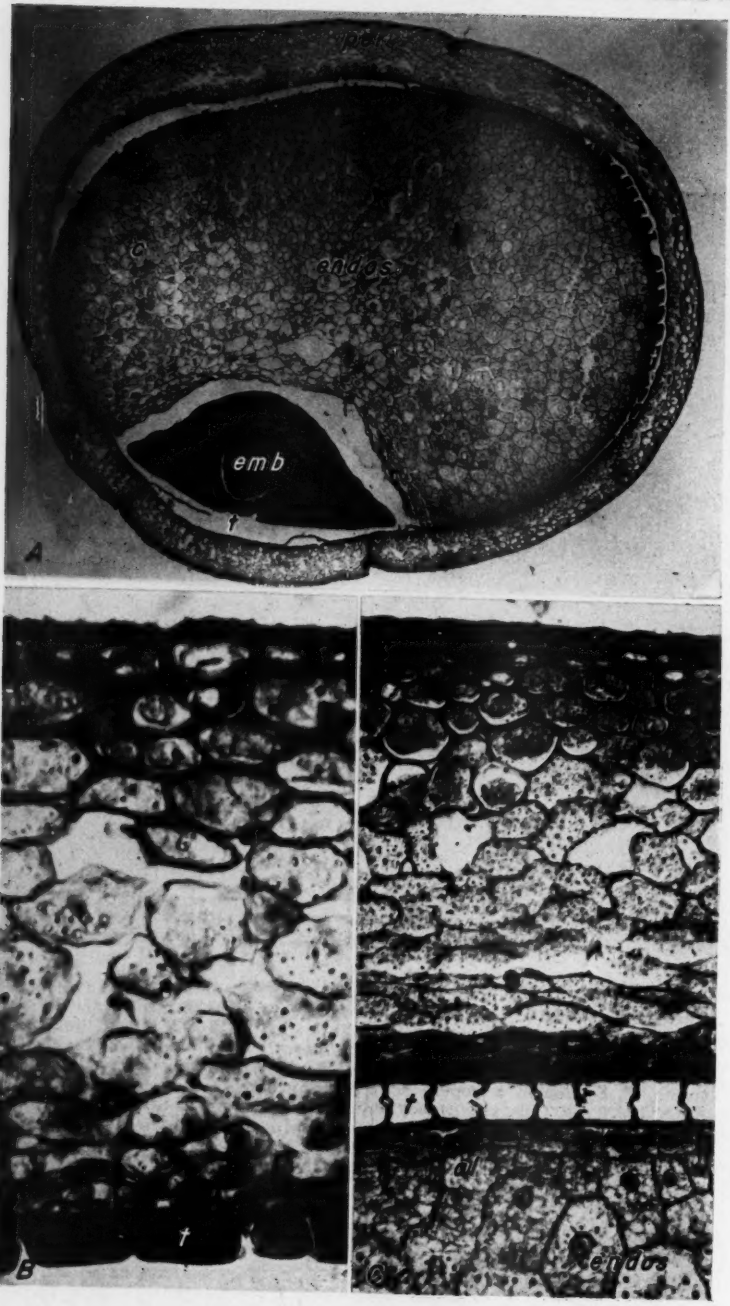
The opening through the inner integument corresponding to the hilum is filled with rows of thin-walled parenchyma cells in which thick-walled fibers differentiate as the seed matures. The outer cell layers are darkly pigmented and correspond to the closing tissue of the hilar orifice described by Harrington and Crocker (*8*) for the caryopsis of Johnson grass.

SEEDLING STRUCTURE

The seedling structure of grasses is well known. That of sorghum has been described in some detail by Sargant and Arber (*15*), and more recently by Reznik (*14*), in connection with studies on homologies.



Cross sections of young embryos: *A*, First nodal, or transaxis, plate. *B*, Horseshoe-shaped procambial bundle (at right), metaxylem vessels, and median-trace bundle (at left). *C*, First nodal plate of embryo 24 hours old. *D*, Section of 24-hour embryo taken slightly above that illustrated in *B*. Scutellum trace already departed and flanges of horseshoe-shaped procambial bundle broken up into four strands. *E*, Section of coleoptile node showing splitting up of median trace. *F*, Section of coleoptile node showing departure of coleoptile traces. All × 110. *colt*, Coleoptile trace; *mt*, median trace; *mx*, metaxylem vessel; *np*, nodal plate; *sct*, scutellum trace.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The structure of the seedling axis between scutellum and coleoptile is uniform except that the tissues in the lower part are mature (pl. 15, *A*) whereas those just below the coleoptile node are meristematic (pl. 15, *B*). The coleoptile node itself (pl. 16, *A*) has a massive central xylem plate from which the elements of the various traces depart. Essentially the vascular tissue of the axis consists of three double groups, which in plate 15, *B*, are designated *a*, *a'*; *b*, *b'*; and *c*, *c'*. Sargant and Arber (*15*) designate the first two of these double groups the lateral traces and the third the median trace. As is shown in an earlier paragraph, the lateral traces supply the traces for the coleoptile and take part in the vascularization of the plumule, while the median trace supplies the principal bundle and some of the laterals of the first foliage leaf.

The vascular tissue of the lower part of the axis (pl. 15, *A*) is surrounded by a thick-walled endodermis of the type found in the root. Within the endodermis are two layers of thick-walled, lignified pericyclic cells. Alternating with the phloem groups and abutting on the pericycle are six relatively large, thick-walled xylem elements with scalariform-pitted walls. They are in continuity with the protoxylem points of the root, but all the protoxylem cells of the axis above the first node are centripetal in relation to the phloem groups, with which they form collateral bundles. The two large metaxylem vessels of the root also are present. Their location is somewhat eccentric between the lateral traces and the median trace (pl. 15, *B*). The portion of the axis just below the coleoptile node contains little xylem and lacks the thick-walled endodermis and pericycle (pl. 15, *B*).

It is generally agreed that the first transaxis plate, with its transversely running procambial strands (pl. 13, *A*, *C*), constitutes the first node. Whether the scutellum, with its solitary endarch bundle, and the coleoptile, with its two traces, constitute the cotyledon and the first foliage leaf is a moot question. Both have a greatly modified leaf structure; and the complicated leaf-trace relationship, especially in the coleoptile, adds confusion to uncertainty.

The axis interval between first node and coleoptile has been variously interpreted as mesocotyl, hypocotyl, and internode. Its structure (pl. 15) deviates so widely from those of root and stem that it cannot be assigned to either category. It resembles the root in having a typical root endodermis, with silica knobs (characteristic of the root endodermis of sorghum and sugarcane), and a jacket of thick-walled pericyclic cells. Also, the two large metaxylem vessels are continuous with those of the root. The protoxylem arches of the root, however, are each reduced here to a single large scalariform-pitted element (pl. 15, *A*). Structural features corresponding to those of the stem are the presence of collateral bundles with prominent phloem and a few scattered centrally located protoxylem elements. This interval, contrary to the opinion of Avery (*2*), does not represent a true transi-

EXPLANATORY LEGEND FOR PLATE 14

Cross sections of mature caryopsis: *A*, Entire caryopsis; × 32. *B*, Pericarp and testa; × 610. *C*, Pericarp, testa, aleurone layer, and peripheral endosperm; × 275. *al*, Aleurone layer; *emb*, embryo; *endos*, endosperm; *per*, pericarp; *t*, testa.

tion in the accepted meaning of the term, especially since it does not, like that of the axis of a corn seedling, contain exarch xylem points alternating with collateral bundles. Elongation is by a meristem situated directly below the coleoptile node (pl. 16, *B*), as, according to Avery (*2*), is the case with corn. Reznik (*14*) has disputed this point, holding that growth in length is from an intercalary meristem located just above the first node and that elongation is acropetal.

Whether the axis interval between the first transaxis plate and the coleoptile should be considered an internode, specifically the first internode, hinges on whether the coleoptile is considered the first foliage leaf. This is an entirely subjective interpretation for which no one has yet presented convincing arguments. Since the unique structure of this interval makes it neither root nor stem or hypocotyl, the term "mesocotyl" is descriptive and more acceptable than the designation "internode."

SUMMARY

Pollen development and macrosporogenesis in sorghum (*Sorghum vulgare* Pers.) are similar to those in other grasses. The synergids degenerate before fertilization. The antipodal nuclei also degenerate early, but the antipodal cells greatly increase in size and spearhead the endosperm into the disintegrating nucellus.

Anthesis begins soon after midnight, but pollen germination is delayed until daybreak. The pollen tube reaches the embryo sac within approximately 2 hours after pollen germination. After fertilization the zygote rests for about 4 hours. The polar-fusion, or primary-endosperm, nucleus divides earlier than the zygote, with the result that four or more endosperm nuclei are formed before the rest period of the egg terminates.

Organ differentiation in the embryo commonly begins on the seventh day and is completed by the twelfth day. The embryo continues to grow until seed is mature. The mature embryo has a complete procambial system but no mature vascular elements. Its various organs are well differentiated, but objective interpretation of the nature of scutellum, coleoptile, and axis interval between first node and coleoptile offers approximately the same difficulties as when workers on embryo and seedling structure in grasses first attempted it more than a century ago.

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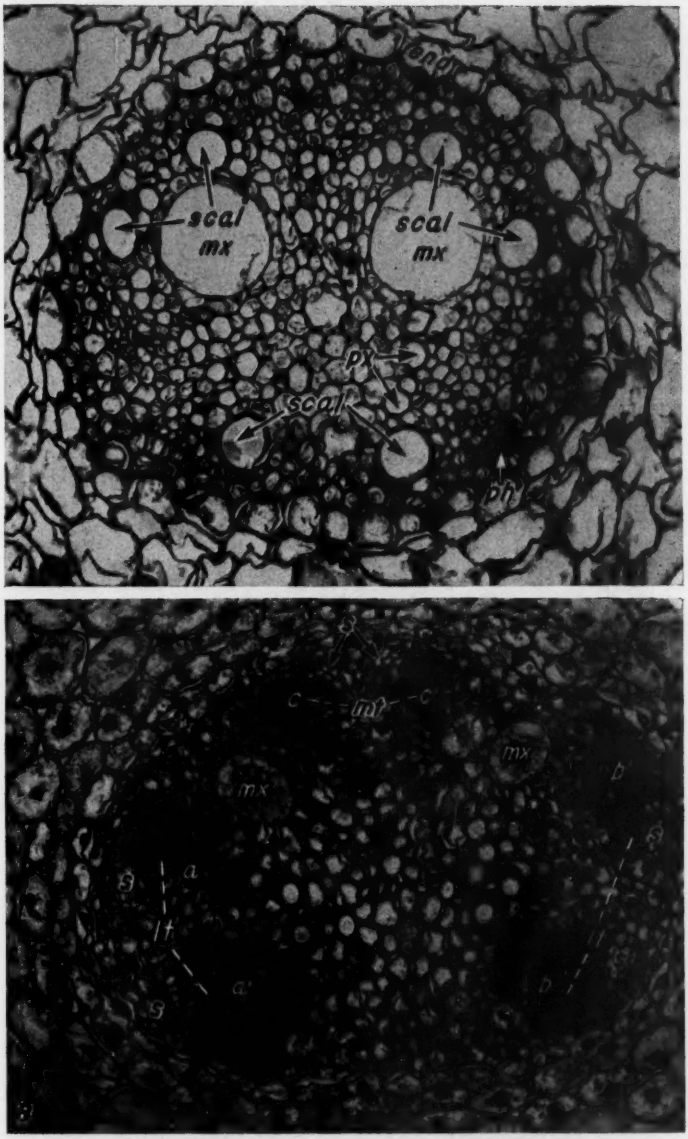

A, Cross section of axis of 8-day-old seedling above divergence of scutellum trace. *B*, Cross section of axis of same seedling just below coleoptile node. Both × 250. *end*, Endodermis; *lt*, lateral traces (*a*, *a'*; *b*, *b'*); *mt*, median trace (*c*, *c'*); *mx*, metaxylem vessel; *ph*, phloem group; *px*, protoxylem; *s*, sieve tubes; *scal*, scalariform-pitted element.

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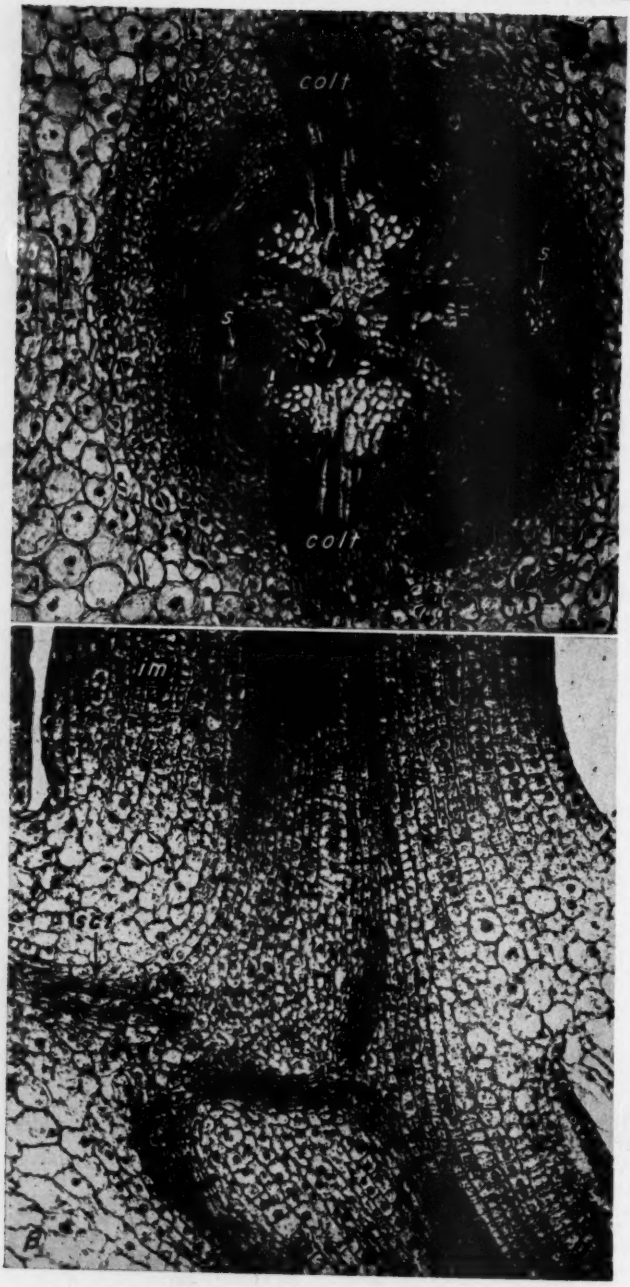
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EXPLANATORY LEGEND FOR PLATE 16

A, Cross section of coleoptile node with massive central xylem plate and peripheral phloem groups. *B*, Longitudinal section of axis of 1-day-old seedling showing departure of scutellar trace (at left) and intercalary meristem (above). Both × 150. *colt*, Coleoptile trace; *im*, intercalary meristem; *s*, sieve tubes of median trace of first foliage leaf; *sct*, scutellar trace.

THE BRAZILIAN CURLY TOP OF TOMATO AND TOBACCO RESEMBLING NORTH AMERICAN AND ARGENTINE CURLY TOP OF SUGAR BEET[1]

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INTRODUCTION

What seems to be the first report of a disease of the curly top type in Brazil is that made by Costa and Forster (*3*)[2] who, in 1939, described a suspected virus disease of tobacco (*Nicotiana tabacum* L.) in the State of São Paulo under the name "encarquilhamento da fôlha." Although attempts to transmit the disease by grafts and by juice inoculation failed, it was suggested, on the basis of symptoms and apparent spread in the field, that the disease might be caused by a virus and that it might be similar to tobacco leaf curl. Two forms, a rugose form and a leaf roll one, were recognized. It now seems well established that the former is a virus disease of the curly top type, whereas the leaf roll condition results from genetic factors.

A disease that now appears to be caused by the virus that produces the rugose form of encarquilhamento da fôlha was described on tomato (*Lycopersicon esculentum* Mill.) by Sauer (*10*) in 1946. This disease causes the leaves of affected plants to roll and the plants to yellow and die. It was shown to be transmitted by the leafhopper *Agallia albidula* Uhl. from diseased to healthy tomato plants. On the basis of symptoms produced on tomato it was suggested that the disease is of the yellows, or curly top, type.

In 1947 Kramer (*7*) extended the known host range of the tomato disease by transmitting it by grafts from tomato to jimsonweed (*Datura stramonium* L.), tobacco (vars. Turkish and White Burley), and petunia (× *Petunia hybrida* Vilm.). He obtained no transmission to these plants or to sugar beet (*Beta vulgaris* L.) by means of dodder (*Cuscuta campestris* Yuncker). Kramer reported also that attempts by Sauer to transmit the disease to sugar beet by means of *Agallia albidula* resulted in no infection.

Since the symptoms described by Costa and Forster (*3*) on tobacco and those described by Sauer (*10*) on tomato are so similar to those produced on these plants by sugar-beet curly top in the United States, it occurred to the writers that the described diseases in Brazil might be due to a single virus and that this virus might be related to that of sugar-beet curly top in the United States. Studies were started at the Instituto Agronômico in Campinas, Brazil, late in 1946 and continued through most of 1947 to obtain further information regarding these suspected relationships. This paper reports the results obtained. For reasons that will be evident, the name "Brazilian curly top" is suggested for the disease.

[1] Received for publication August 24, 1948.

[2] Italic numbers in parentheses refer to Literature Cited, p. 692.

GEOGRAPHIC DISTRIBUTION

The known distribution of Brazilian curly top is somewhat limited. Apparently it occurs throughout the State of São Paulo where tomatoes and tobacco are grown. Of the "municípios" in which Costa et al. (*4*) reported occurrence of encarquilhamento da fôlha on tobacco in 1942, records show that the rugose form was identified in Bragança, Iacanga, Piracicaba, São Bento do Sapucaí, Socorro, and Tietê. Whether occurrence of the disease is coincident with that of the vector, which seems to have a widespread distribution throughout tropical America, remains to be determined.

The conspicuous nature of the disease on both tobacco and tomato and the fact that it was discovered relatively recently suggest that it may have been present in cultivated crops in the State of São Paulo only a few years. However, since it does not occur abundantly on tobacco and there is danger of confusing its symptoms with those of other virus diseases on tomato, it is difficult to judge whether it is a new disease or has been present for a considerable number of years without discovery.

HOST RANGE OF BRAZILIAN CURLY TOP

PLANTS INFECTED NATURALLY

Tomato and tobacco are the two plants of greatest economic importance attacked by Brazilian curly top. The annual loss from the disease probably is not very large in the State of São Paulo, but in individual plantings of tomato as many as 10 to 20 percent of the plants are attacked in some seasons. In experimental plantings of tomato made on the farm of the Instituto Agronômico, near Campinas, the disease has been more noticeable during the winter. It has not been determined whether this is due to a higher incidence during the winter or to a partial masking in the summer by "vira-cabeça" (spotted wilt), which kills many of the plants early in the season before symptoms of curly top develop. However, it might be expected that the incidence of disease would be higher during the winter, since during this period much of the weed cover dries and there is a greater migration of leafhoppers from weed hosts to cultivated fields.

Although the disease on tobacco is rather widely distributed, it rarely attacks a very high percentage of plants. As many as 6 percent of some plantings have shown infection, but more often the disease is limited to a few plants per acre. The amount of injury depends somewhat on the age of the plant at the time of infection, but if the disease appears early the yield and quality of the leaf are greatly reduced.

The disease was found in one instance in a field of buckwheat (*Fagopyrum esculentum* Moench), but little is known about its prevalence on this crop.

The most important weed host so far as known is spiny bur, or "carrapicho de carneiro" (*Acanthospermum hispidum* DC.). This plant occurs in considerable abundance in cultivated areas. During the summer of 1946–47 it was found to be infected in several locations near Campinas. In some areas where there was a more or less solid stand, about 3 percent of the plants were found to be diseased. Since this weed is also an excellent host plant for *Agallia albidula*, it seems prob-

able that it is one of the important sources of virus for infection of tobacco and tomato fields.

PLANTS INFECTED EXPERIMENTALLY

More than 40 species and varieties of plants were inoculated with Brazilian curly top by means of the leafhopper vector, *Agallia albidula*. The sources of virus for these inoculations and the number of leafhoppers that fed on each plant varied greatly with the different species and varieties. Numerous viruliferous leafhoppers were used on plants that were difficult to infect, such as tobacco, and fewer on those that proved to be more susceptible. The results of representative tests are shown in table 1.

TABLE 1.—*Results of tests of plants for susceptibility to Brazilian curly top and a hosts for reproduction and survival of Agallia albidula, the vector of the curly top virus*

Plants tested	Plants—		Progeny produced by leafhopper	Survival of adult stock of leafhopper
	On which viruliferous leafhoppers fed	Infected		
	Number	*Number*		
Acanthospermum hispidum (spiny bur)	50	20	Many	Excellent.
Amaranthus caudatus L.	5	0	do	Do.
Amaranthus retroflexus L. (pigweed)	2	0	do	Do.
Atriplex semibaccata R. Br. (Australian saltbush)	31	0	do	Do.
Beta patellaris Moq	4	0	None	2–5 days.
Beta vulgaris (sugar beet): [1]				
R. & G. Old Type	41	10	do	Do.
S. L. 68	20	1	do	Do.
S. L. 842	20	0	do	Do.
U. S. 15	21	1		Do.
U. S. 33	19	2		Do.
U. S. 222	20	0		Do.
Callistephus chinensis (L.) Nees (China-aster)	7	0	Many	Excellent.
Chenopodium album L. (lambsquarters)	2	0	Few	Good.
Coffea arabica L. (coffee)	5	0	None	5–14 days.
Datura stramonium (jimsonweed)	25	7	Many	Excellent.
Datura sp. (triple-corolla type)	6	0	do	Do.
Erigeron bonariensis L. (fleabane)	1	0	Several	Do.
Euphorbia prunifolia Jacq. (amendoim)	18	9		
Fagopyrum esculentum (buckwheat)	20	16	Many	Excellent.
Gossypium hirsutum L. (upland cotton)	5	0	None	Poor.
Lactuca sativa L. (lettuce)	3	0	Many	Fair.
Linum lewisii Pursh (prairie flax)	29	2		
Linum usitatissimum L. (flax):				
Calor	8	5		
Rocket	34	20		
Lycopersicon esculentum (tomato)	54	6	None	Poor.
Nicotiana glauca Graham (tree tobacco)	5	0	do	Do.
Nicotiana glutinosa L.	10	1	do	Do.
Nicotiana tabacum (tobacco)	98	7	do	2–5 days.
Oxalis spp.:				
Large-leafed species	3	0	do	Fair.
Small-leafed species	3	2	do	Do.
× *Petunia hybrida* (petunia)	2	0	do	Poor.
Phaseolus vulgaris L. (field bean var. Red Mexican).	15	0	do	2–5 days.
Phyllanthus corcovadensis Muell. Arg.	2	2	do	Fair.
Phytolacca americana L. (pokeweed)	22	0	Few	Do.
Portulaca oleracea L. (common purslane)	3	0	Many	Good.
Sida rhombifolia L.	4	0	Few	Do.
Solanum nigrum L. (common nightshade)	2	0	Many	Do.
Solanum tuberosum L. (potato)	9	0	do	Excellent.
Spinacia oleracea L. (spinach)	25	20	do	Good.
Stellaria media (L.) Cyr. (chickweed)	27	20	do	Do.
Vinca rosea L. (periwinkle)	3	0	None	Poor.
Zinnia elegans Jacq. (zinnia)	9	1		

[1] Varieties released for commercial use by the Bureau of Plant Industry, Soils, and Agricultural Engineering are designated as "U. S." varieties. "S. L." refers to varieties maintained at the U. S. Sugar Plant Field Station at Salt Lake City, Utah.

Even in the seedling stage sugar beet appears to be very resistant to infection. Tomato also shows considerable resistance to infection. In 1 test with potted plants about 8 cm. tall in which 1 viruliferous leafhopper was allowed to feed 7 days on each plant, only 3 of 83 plants became infected. Other tests with larger numbers of leafhoppers also gave low percentages of infections.

There is evidence that tobacco is relatively susceptible to infection when the plants are very small, but its resistance increases rapidly as the plants pass from the seedling stage. In a test in which 20 tiny seedlings were inoculated by means of 1 viruliferous leafhopper per plant, 5 became infected. However, no infection was obtained by means of single leafhoppers on plants with 6 to 10 leaves, and only a low percentage of infection was obtained on plants of this size on which larger numbers of insects fed. In tests in which 10 to 50 viruliferous leafhoppers were allowed to feed on tobacco plants with 6 to 10 leaves until the leafhoppers died, only 2 of 29 plants became infected. Of 24 plants with 6 to 10 leaves that were inoculated in the field by means of 5 viruliferous leafhoppers per plant, none became infected. This difficulty in producing infection under greenhouse and field conditions is somewhat surprising in view of the fact that as many as 6 percent of the plants occasionally are infected in the field.

The fact that tobacco and tomato appear to be very resistant to infection when inoculated by means of *Agallia albidula* and that the leafhopper survived on these plants for only a short time suggested the possibility that part of the natural spread might be due to the presence of a second vector. However, tests made in attempts to transmit the disease by means of several other species of leafhoppers have given no infection.

Some of the species of plants, including *Amaranthus* sp., *A. caudatus*, *Callistephus chinensis*, *Chenopodium album*, *Erigeron bonariensis*, *Lactuca sativa*, *Sida rhombifolia*, *Solanum nigrum*, and *S. tuberosum*, that did not show symptoms in previous tests were tested further for susceptibility. In these tests vigorous plants 10 to 20 cm. tall were selected, large cages were placed over each plant, and 50 viruliferous leafhoppers were introduced into each cage and allowed to feed more than 40 days. None of the plants developed symptoms of disease. Nymphs were produced on all of these plants. When the nymphs were about half grown, some of them were transferred from each plant to suitable test plants of *Datura stramonium*, *Fagopyrum esculentum*, or *Stellaria media* to determine whether they were viruliferous. None of these test plants became infected, and it is assumed that the nymphs were unable to acquire virus from the plants on which they hatched. It seems probable, therefore, that all of the species tested for susceptibility in this experiment are extremely resistant to or immune from infection by the Brazilian curly top virus.

In a series of transfer tests made to study symptoms produced by virus from different sources, plants of tobacco, tomato, and spiny bur showing natural infection in the field were selected as sources of inoculum for cross-transfer tests. Nonviruliferous leafhoppers from stock colonies were allowed to feed on plants infected by virus from each of the three field sources and then were transferred to healthy plants of various species. In this manner the virus was transmitted

from diseased to healthy plants of tobacco, tomato, and spiny bur, respectively, and from each of these to healthy plants of the other two species. Also the virus was transmitted from tobacco to buckwheat, chickweed, and jimsonweed; and from tomato and spiny bur to buckwheat, chickweed, and spinach. No differences were noted in the symptoms produced by virus from the three sources on any of the species of test plants. These tests seem to prove conclusively that the rugose form of encarquilhamento da fôlha disease of tobacco described by Costa and Forster (*3*), the tomato disease described by Sauer (*10*), and the leaf-curling disease found occurring naturally on spiny bur are caused by the same virus.

DESCRIPTION OF SYMPTOMS ON VARIOUS HOSTS

TOBACCO

The first symptoms of Brazilian curly top on tobacco appear on the young leaves and consist of vein clearing and bending downward of the leaf tips and margins. As the leaves enlarge they become rolled and crinkled because of the restriction of growth of the larger veins. The veins become swollen irregularly and distorted. Under greenhouse conditions symptoms tend to become less severe as the plant matures, but usually symptoms are conspicuous on the flower parts and often both calyx and corolla are deformed because of the irregular growth and swelling of the main veins.

In the field leaves of diseased plants are curled downward, appear thick and brittle, and are smaller and more rugose than those of normal plants (fig. 1). Most plants observed have remained stunted, but there is evidence of a certain amount of recovery from the more severe early symptoms of the disease in some instances. A rather

FIGURE 1.—Tobacco plants of the variety Amarelinho photographed at the Estação Experimental de Tietê of the Instituto Agronômico, State of São Paulo, Brazil, 1938: *A*, Healthy plant; *B*, plant affected with Brazilian curly top.

high degree of recovery was noted in field plants pruned back and transplanted to pots in the greenhouse. The first leaves produced on the suckers of these plants were dwarfed and rolled; soon, however, others that were more or less normal in size and shape were produced. In some plants recovery was almost complete, and the most conspicuous symptoms consisted of vein clearing in the younger leaves. It is of interest that this type of reaction is characteristic also of tobacco affected with North American curly top.

TOMATO

About 15 to 20 days after infection tomato plants begin to produce young leaves that show a certain amount of vein clearing and are twisted or otherwise distorted and rolled upward (fig. 2). Growth is markedly retarded, and soon the older leaves become dull and somewhat rolled. Later they appear thick and brittle and take on various shades of yellow, often tinged with purple. Figure 3, *A*, *b*, shows

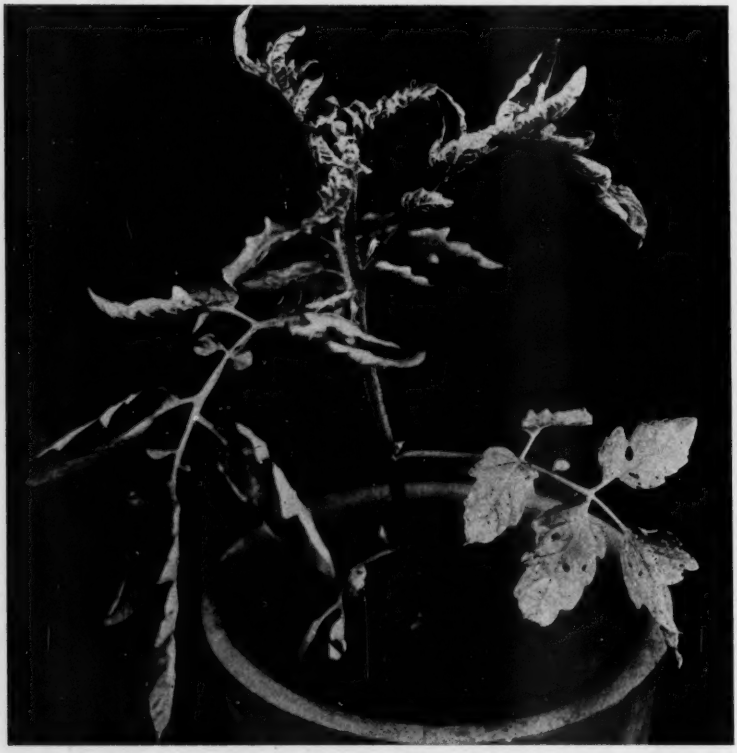

FIGURE 2.—Small tomato plant showing early stage of Brazilian curly top characterized by rolling and twisting of young leaves.

a diseased plant with typically rolled leaves and a generally stunted appearance. Diseased plants soon cease to produce fruit and eventually die. In general appearance plants affected with Brazilian curly top resemble to some degree those attacked by vira-cabeça (spotted wilt), but they can be distinguished readily from such plants by the absence of necrotic spots on the leaves.

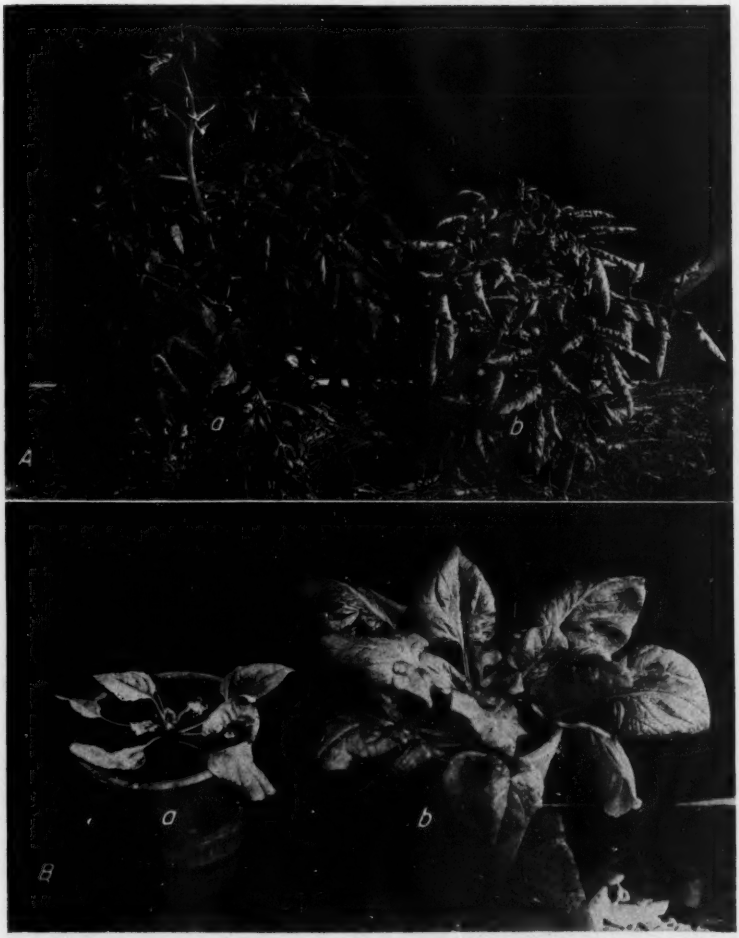

FIGURE 3.—*A*, Healthy (*a*) and diseased (*b*) plants of tomato under field conditions. The latter plant shows typical stunting and leaf rolling. *B*, Diseased (*a*) and healthy (*b*) plants of spinach of the same age. The diseased plant was small when infected, has produced very little growth, and shows typical dwarfing and leaf curling.

SPINY BUR

Symptoms become evident in young spiny bur plants about 10 to 15 days after infection. First symptoms consist of increased bluntness and slight turning downward of half-grown leaves. Young leaves may show inconspicuous vein clearing and a slight amount of vein swelling, but they are not deformed. Growth of the plant is markedly retarded, and leaves roll upward, turn yellow, and die prematurely. Diseased plants in the field are conspicuous because of their general yellow appearance, rolled leaves, and stunted condition. These symptoms are very similar to those produced on spiny bur by North American curly top.

SPINACH

Symptoms begin to appear on young spinach plants about 10 days after infection. The young leaves show conspicuous vein clearing of the type illustrated in figure 4, *B*. Vein clearing is very similar to that produced on spinach by North American curly top (fig. 4, *C*, *a*). Growth is markedly retarded, the few leaves produced are small and badly distorted and twisted, and the larger veins are swollen irregu-

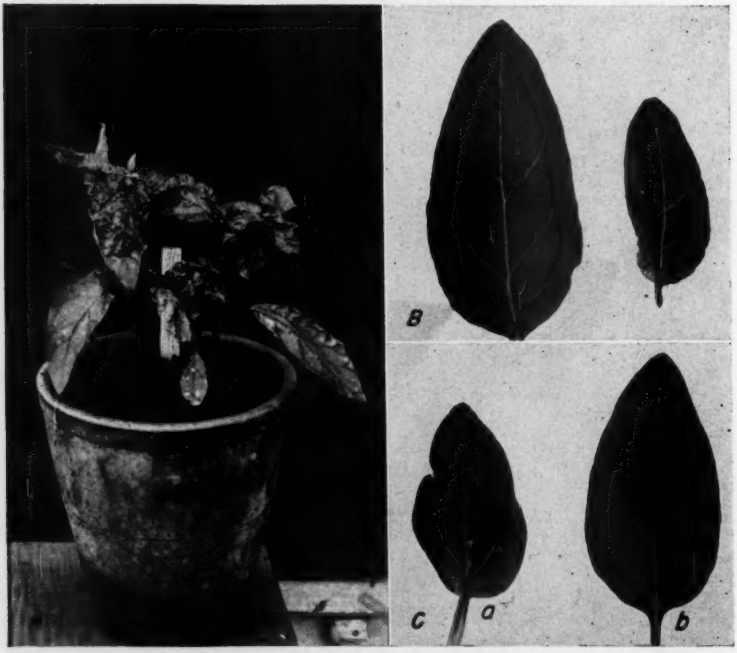

FIGURE 4.—*A*, Plants of jimsonweed affected with Brazilian curly top, showing stunting, leaf curling, and flower deformities. *B*, Leaves of spinach showing type of vein clearing produced by Brazilian curly top. *C*, Leaf (*a*) of spinach showing vein clearing produced by North American curly top and leaf (*b*) from healthy plant.

larly. Soon the older leaves produced before infection begin to yellow, and the plants remain stunted and yellow until they succumb to the disease. The severe effects of the disease on plants infected when small are illustrated in figure 3, *B*, *a*. Larger plants may live for several weeks, but growth is arrested and the plants are stunted and conspicuously pale or yellow.

FLAX

On susceptible varieties of flax the first evidences of disease consist of vein swellings on the younger leaves, often resulting in marked leaf distortion. The tips of the stems curve and turn downward. In the region of curvature and outward toward the tip, the stem becomes rough and swollen and frequently turns red. All leaves produced after infection are distorted, and there are swollen areas along the veins. Plants produce very little additional growth after they become diseased; soon the plant yellows, some of the leaves wilt and drop, and the plant dies.

The Calor and Rocket varieties proved to be very susceptible to infection and were severely injured. Prairie flax appears to be much more resistant to infection and to be injured less severely when it becomes diseased.

BUCKWHEAT

Buckwheat is very susceptible to infection, especially in the seedling stage, and is severely injured by the disease. The younger leaves show distinct vein clearing; older leaves are badly twisted and distorted and frequently are rolled upward so that a part of the dorsal surface is exposed when viewed from above. Portions of the stems, especially those bearing the flower parts, may be crooked and swollen irregularly. Growth is retarded, and the plants are markedly dwarfed. If the plants are small when infected, they usually die before they produce seed.

SUGAR BEET

On sugar-beet plants the disease first produces a type of vein clearing that may be evident in most of the veins of young leaves throughout their entire length, but sometimes the cleared areas appear as circular spots or short lines. This vein clearing is indistinguishable from that produced on sugar beet by North American curly top. Vein clearing is accompanied by a certain amount of rugoseness and downward rolling of the leaf edges or by leaf twisting. As the leaves mature, the cleared areas turn a normal green and the veins become swollen and irregular. As the plant increases in size the symptoms become less severe. On older plants they consist chiefly of vein swellings and irregularities most evident on the under side of the leaf (fig. 5, *A*). Sometimes spinelike outgrowths a millimeter or more in length are produced (fig. 5, *B*). In the more severely affected plants the upper surface of the leaf may show a certain amount of rugosity, probably resulting from the restricted growth of the veins. The leaves, however, are more or less normal in shape and size, and the plants show very little stunting.

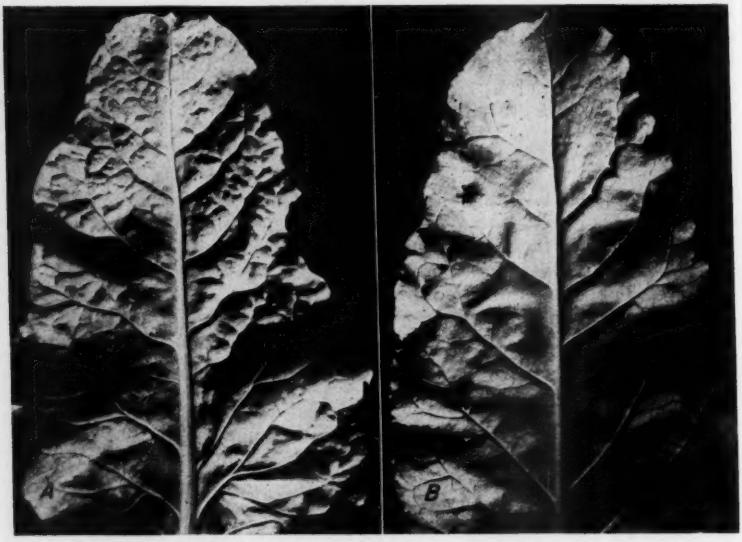

FIGURE 5.—*A*, Leaf of sugar beet affected with Brazilian curly top, showing vein swellings and irregularities associated with a certain amount of rugosity on the lower and upper surfaces. *B*, Leaf showing spinelike outgrowths from the veins of the undersurface.

JIMSONWEED

The first symptom of Brazilian curly top on plants of jimsonweed is vein clearing in the young leaves. The first leaves that show this characteristic are not curled or distorted, but they do not grow to normal size. After these leaves mature, the larger veins appear as almost white lines when viewed from above. Leaves produced later are small, twisted, and otherwise distorted (fig. 4, *A*). The veins are swollen and very irregular. Stems of the new growth have short internodes. Blossoms are small and distorted with swellings along the veins of the calyx and corolla. The corolla is sometimes wrinkled both longitudinally and transversely. Blossoms usually drop before opening. Plants may live indefinitely and are a deeper green than normal ones.

OXALIS

A small-leafed species of *Oxalis* common in cultivated areas near Campinas is susceptible to Brazilian curly top; a common species with larger leaves appears to be immune. On diseased plants the leaflets are smaller than normal and are distorted and twisted; the veins may be twisted or abruptly curved, producing bulges in the leaf, and sometimes they have spinelike outgrowths. The petioles and stems are distorted and have outgrowths, often reddish in color, varying from small elevations to conspicuous enations 5 mm. or more long, and frequently restricted to one side. The blossoms and seed pods show vein swelling and distortion. The plants do not turn yellow and are not so severely injured as certain other susceptible species.

CHICKWEED

Chickweed is very susceptible to infection and is severely injured. About 10 to 15 days after young plants are infected, leaves at the growing tips roll upward and become twisted and otherwise distorted. Sometimes on the main veins appear swollen areas which cause the leaflets to be curved abruptly. Growth is much retarded, the older leaves turn yellow, and the plant dies.

TRANSMISSION OF BRAZILIAN CURLY TOP

JUICE INOCULATIONS

Seedling plants of buckwheat were inoculated with juice from diseased spinach and chickweed plants. In these inoculations the seedling plants were first sprinkled with an abrasive and then the leaves and cotyledons were rubbed with a cloth pad saturated with juice. Immediately afterward the juice was washed from these plants with a fine spray. Similar inoculations were made on plants of tobacco and sugar beet. No infection resulted from any of these inoculations.

TRANSMISSION BY GRAFT

Since Kramer (*7*) had already shown that Brazilian curly top is transmissible by graft, only limited additional tests were made; these were designed primarily to compare the effects of the virus from different localities and hosts. In these tests the disease was transmitted from tomato to tomato and tobacco and from tobacco to tobacco and tomato. So far as observed the source of inoculum had no effect on the characteristics or severity of the symptoms. It was noted, however, that when infected scions were placed in the sides of stems of tobacco plants frequently the plants continued to grow and blossom without showing symptoms of the disease. When the plants were pruned back and the diseased scion was grafted to the top of the stem, the growth from the lateral buds showed symptoms. In both cases production of symptoms, however, required a longer time than is required for the production of symptoms on tobacco by North American curly top under similar conditions. This evidence indicates that invasion of tobacco plants by the virus of Brazilian curly top may be somewhat slower than that by the virus of North American curly top.

TESTS WITH INSECTS

Transmission tests were made with a number of species of leafhoppers other than *Agallia albidula*. In general, in these tests plants of various kinds were swept with an insect net and the entire content of the net was put in a cage that was placed over diseased spiny bur plants. After the insects had fed 1 to 3 days on the diseased plants, they were transferred to healthy seedling plants of spiny bur. In some tests the species of leafhoppers were not separated; in others each species was separated out and placed in leaf cages singly or in groups of two to five on small, healthy plants. Because no infection was obtained in any of these experiments, only one of the species of leafhopper was submitted for identification. This proved to be *Agalliana*

ensigera Oman,[3] the vector of Argentine curly top virus. Limited transmission tests were made with this species. In one experiment leafhoppers collected from the field were allowed to feed on diseased spiny bur plants 3 days and then were transferred to small, healthy tomato and spiny bur plants. Five leafhoppers were allowed to feed on each healthy plant. Of the seven plants of each species on which the leafhoppers fed, none became infected. In other tests in which rather large numbers of leafhoppers were collected from the field and placed on healthy plants of spiny bur, no infection was obtained.

CHARACTERISTICS, DISTRIBUTION, AND HOST RANGE OF THE VECTOR

The vector of the Brazilian curly top virus is a pale yellowish leafhopper (*Agallia albidula*) about 3.5 mm. long in the adult stage and marked with light brown.

According to Oman (*8*) this leafhopper is a common species in the Tropics of the Western Hemisphere. It has been reported from Cuba, Isla de Pinos, Puerto Rico, Dominican Republic, St. Vincent Island, St. Thomas Island, Trinidad, and the States of Rio de Janeiro, Minas Geraes, Matto Grosso, Pernambuco, and Amazonas, Brazil. It was distributed generally around Campinas in the State of São Paulo, Brazil, during the summer of 1946–47 and through the following fall, winter, and spring. Probably the insect reaches its highest population density during December, January, and February, but this may depend to a large degree on the distribution of the rainfall and the host plants available.

The insect was found on a wide range of host plants, including *Acanthospermum hispidum*, *Portulaca oleracea*, *Amaranthus* spp., *Chenopodium album*, tomato, potato, sweet orange (*Citrus sinensis* (L.) Osbeck), and several species of grasses. Two important weed breeding plants appear to be *A. hispidum* and *P. oleracea*.

In order to obtain further evidence concerning the range of plants on which the insect is able to reproduce, an experiment was made in which plants of various types were placed in breeding cages kept in a greenhouse and 50 leafhoppers were put in each cage. Notes were taken on the time of appearance and the numbers of nymphs produced. At favorable temperatures nymphs began to hatch on some plants in about 14 days, and the first generation matured about 40 days from the time the eggs were deposited. The plants on which leafhoppers were colonized are shown in table 1. Of these plants, *Acanthospermum hispidum*, *Portulaca oleracea*, *Amaranthus caudatus*, *Datura stramonium*, potato, and *Solanum nigrum* appeared to be excellent hosts for rearing the leafhopper. Several others, including *Sida rhombifolia* and *Chenopodium album*, were less favorable, but the survival of the original colony of leafhoppers was good. On several plants, notably sugar beet, tomato, tobacco, *Beta patellaris*, and Red Mexican variety of field bean, the insect did not breed and survived only a short time.

[3] The authors are indebted to P. W. Oman, of the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, for identification of this insect.

THE VIRUS

INCUBATION PERIOD IN VECTOR

Tests were made to determine the period that must elapse between the beginning of feeding of leafhoppers on diseased plants and the time they become able to transmit the virus to healthy plants. In these tests nonviruliferous leafhoppers were allowed to feed for periods of 2 to 24 hours on diseased plants. After feeding they were caged on healthy seedling plants of buckwheat and at intervals were transferred to fresh lots of seedling plants. Table 2 shows the results of four of these tests. In test 2, one plant was infected in the 6-hour incubation series, but since no infection was obtained in the 8-, 24-, and 48-hour incubations in the same test this result may not be reliable. With this exception the incubation period as indicated by the results of these four tests appears to be 24 hours or more. In test 3, one plant was infected that was inoculated by means of leafhoppers in which the virus had a maximum incubation period of 24 hours. However, more infection was obtained in the 48- and 96-hour periods, indicating that the infectivity of the leafhoppers increased with time up to at least 48 hours.

TABLE 2.—*Incubation period of the Brazilian curly top virus in Agallia albidula*

Test No.	Period of leafhopper feeding on diseased plants	Buckwheat plants inoculated and infected by means of leafhoppers in which the virus had an incubation period of—													
		2 hours		4 hours		6 hours		8 hours		24 hours		48 hours		96 hours	
		Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
	Hours	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
1	1	8	0	8	0	8	0	8	0	8	0	8	0	8	0
	2	8	0	8	0	8	0	8	0	8	0	8	1	8	1
	3	8	0	8	0	8	0	8	0	8	0	8	0	8	0
2	4					8	1	8	0	8	0	8	0	8	0
	6					8	0	8	0	8	0	8	0	8	1
	8									8	0	8	0	8	2
3	6							24	0	24	0	24	6	24	6
	16									12	1	12	2	12	2
4	[1] 24											[1] 24	4		

[1] Of 24 plants inoculated by means of leafhoppers in which the virus had an incubation period of 72 hours 9 became infected.

PERIOD OF RETENTION IN VECTOR

Two methods were used in attempts to determine the time viruliferous leafhoppers will retain the virus of Brazilian curly top when they are not permitted to replenish their supply of virus by feeding on diseased plants.

In the first of these tests, leafhoppers that had been reared on diseased spiny bur plants until they were about half grown were caged singly on seedling plants of spiny bur. Throughout the remainder of their lives they were transferred daily except Sunday to fresh seedling plants of this species. It was assumed that leafhoppers would not infect the test plants and recover virus during the 1- or 2-day feeding

period. The results obtained with 16 of these leafhoppers are shown in table 3. From the number of plants infected during 37 transfers it is evident that the virus is retained for considerable periods. It is doubtful whether there is evidence of depletion of virus content of the leafhoppers over the period shown. However, leafhopper 9 lived through 7 additional transfers (not shown in table 3) and did not transmit, making 28 successive transfers without transmission after transmitting to 6 plants of the first 16 transfers. Also, leafhopper 7 survived through 44 additional transfers (not shown in table 3), transmitted on the forty-fourth and fifty-seventh transfers, and then failed to transmit in the next 24 successive transfers. These results indicate that it may be possible to exhaust the store of virus in leafhoppers by successively transferring them to fresh healthy plants, provided the insects live long enough. This would indicate that the virus is not able to maintain its original concentration in the leafhopper under such conditions and is evidence against multiplication of the virus in the leafhopper.

The variation in the number of plants infected by the different leafhoppers in these tests indicates a wide difference in ability of individuals to transmit. This is characteristic also of *Circulifer tenellus* (Baker) (*9*),[4] the vector of North American curly top virus.

In a second test large nymphs and young adult leafhoppers were transferred from diseased plants to those of species thought to be immune from curly top. After feeding on the immune plants the original leafhoppers were removed at intervals and placed in leaf cages on healthy seedling plants of susceptible species. In each test nymphal progeny of the viruliferous leafhoppers were also transferred to the plants of susceptible species. The results of these tests are shown in table 4. They give no evidence as to whether there was a decrease in virus content of the leafhoppers during their periods of feeding on immune plants, but they do show that some of the leafhoppers were able to retain virus for periods ranging from 42 to 85 days. The fact that the progeny of these leafhoppers were unable to transmit the disease when transferred from the breeding plants to healthy seedling plants of susceptible species indicates that the plants from which the insects were taken are immune from Brazilian curly top and also that the virus is not transmissible through the egg of *Agallia albidula*.

No extensive studies of the properties of the virus of Brazilian curly top have been made. In a preliminary experiment the leafhopper vector was allowed to feed on liquid preparations of alcoholic precipitates of crushed viruliferous leafhoppers and of juice of diseased plants of spinach and spiny bur and then caged singly on small plants of chickweed and spinach. In one test infection was obtained from the juice of spinach by this method. Since the precipitation was carried out in 50-percent alcohol, this test shows that the virus is not inactivated by this concentration of alcohol in short exposures and that the virus is carried down in the alcoholic precipitate of spinach juice and can be resuspended in water and taken up in the feeding of nonviruliferous leafhoppers. One test to determine the thermal inactivation point of the virus in which liquid preparations were made from

[4] This leafhopper was formerly called *Eutettix tenellus* (Baker), but recently it was transferred to the genus *Circulifer* by Oman (*9*).

TABLE 3.—*Transmission of curly top by Agallia albidula to small plants of spiny bur in successive transfers (daily except Sunday)*

| Leafhopper No. | Transmission [1] on indicated successive transfers | | |

TABLE 4.—*Retention of virus by Agallia albidula during feeding on immune plants*

Immune plant	Time on immune plant before testing	Susceptible plants inoculated and infected—			
		By original viruliferous leafhoppers		By progeny of viruliferous leafhoppers	
		Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Days	*Number*	*Number*	*Number*	*Number*
Amaranthus caudatus	51	10	2	10	0
Amaranthus sp.	46	10	4	10	0
Callistephus chinensis	78	4	1	4	0
Chenopodium album	59	10	2	10	0
Lactuca sativa	85	4	1	4	0
Sida rhombifolia	42	10	4	10	0
Solanum nigrum	68	4	0	4	0

alcoholic precipitates of juice of diseased plants of spinach and heated 10 minutes at 5° intervals from 60° to 80° C., inclusive, gave infection only in the test plants inoculated with the unheated preparation. It is not considered, however, that the thermal inactivation of the virus is below 60°, since at times similar results are obtained with the North American curly top virus, which is known to have a thermal inactivation point between 75° and 80°.

DISCUSSION

RELATION OF BRAZILIAN CURLY TOP VIRUS TO NORTH AMERICAN AND ARGENTINE VIRUSES

One of the striking features of Brazilian curly top is its close resemblance to North American curly top on the host plants on which it has been observed. So far as determined the host range of the Brazilian curly top virus (table 1) is the same as that of the North American virus. This parallelism extends even to the severity of symptoms on most of the host plants. For example, both viruses produce severe symptoms on tomato, tobacco, jimsonweed, chickweed, flax, spinach, and buckwheat. On these hosts it is doubtful whether the two diseases could be separated on the basis of symptoms. Potato, Red Mexican variety of field bean, *Chenopodium album*, *Atriplex semibaccata*, and *Beta patellaris* are very resistant to or immune from both of these viruses. On tobacco both viruses produce severe symptoms at first; then the plants recover to a considerable degree, but the recovery appears to be more marked with North American curly top. The Calor and Rocket varieties of flax are very susceptible to Brazilian curly top, but prairie flax is resistant. A similar reaction of these three types of flax to North American curly top was reported by Giddings (*5*).

The greatest difference in host reaction to the two viruses was encountered in sugar beet. This plant is very susceptible to North American curly top, and certain varieties are injured severely. All varieties tested, including two that are very susceptible to North American curly top, appear to be resistant to infection and to injury by the Brazilian virus. However, R. & G. Old Type, a variety that is very susceptible to North American curly top, appears to be more

susceptible to infection by the Brazilian curly top virus than are certain varieties resistant to North American curly top.

The similarities between Argentine curly top and North American curly top have been pointed out (*1*). The Argentine curly top differs from the Brazilian type in known host range chiefly in the failure of the Argentine virus to attack tomato and tobacco.

It was suggested that curly top in Argentina and in North America is produced by a complex of related virus strains and varieties (*1*). The Argentine virus was considered to be a variety of this complex. In some respects Brazilian curly top is even more similar to North American curly top than is the Argentine disease. In view of these similarities it seems logical that the Brazilian curly top should be considered related to similar diseases that occur in North America and Argentina and that for the present at least the causal virus should be classified as a variety of the curly top virus complex of the Western Hemisphere.

In his system of nomenclature of plant viruses Holmes (*6*) designated the sugar-beet curly top virus *Chlorogenus eutetticola* Holmes. Later Carsner and Bennett (*2*) suggested that the virus falls more logically in the genus *Ruga* of Holmes and renamed the virus *Ruga verrucosans*. The Argentine curly top virus was considered to be a variety of North American curly top virus and was given the variety name *distans* (*1*). Since the Brazilian virus appears also to be a variety of the curly top complex, it is suggested that it be given the variety name *brasiliensis* because it was discovered in Brazil and, so far as now known, is limited to that country.

PROPOSED NAME AND DESCRIPTION OF THE BRAZILIAN CURLY TOP VIRUS

Ruga verrucosans Carsner and Bennett var. ***brasiliensis*** n. var.

Virus causing severe disease of *Lycopersicon esculentum* and *Nicotiana tabacum* in the State of São Paulo, Brazil. Occurring naturally also on *Fagopyrum esculentum* and *Acanthospermum hispidum*, the latter probably one of the chief sources of virus in nature. Cause of severe injury to *Datura stramonium*, *Linum usitatissimum*, *Oxalis* spp., *Spinacia oleracea*, *Stellaria media*, and *Zinnia elegans*. *Beta vulgaris* susceptible to infection but apparently very resistant to injury. The induced disease characterized chiefly by vein clearing, swelling, and distortion, by leaf rolling and crinkling, and by dwarfing and sometimes yellowing of affected plants. Virus transmitted under natural conditions by *Agallia albidula*. Persisting in the vector up to 85 days when feeding on immune plants, but not transmitted through the eggs of the vector. Resistant to short exposures to 50-percent alcohol, carried down in precipitate of spinach juice in 50-percent alcohol, and recoverable from the precipitate. Other properties unknown.

Type locality.—Município of Campinas, State of São Paulo, Brazil.

SUMMARY

Evidence is presented indicating that the rugose form of the "encarquilhamento da fôlha" disease of tobacco described by Costa and Forster (*3*) and a disease of tomato described by Sauer (*10*) are caused by the same virus. In addition, this disease was found on buckwheat (*Fagopyrum esculentum*) and spiny bur (*Acanthospermum hispidum*), the latter a common weed of cultivated fields. It was transmitted by means of the leafhopper *Agallia albidula* to a wide range of plants including tobacco, tomato, spinach, sugar beet, flax, jimsonweed, buckwheat, spiny bur, oxalis, zinnia, and chickweed.

The symptoms on these plants are very similar to those produced by sugar-beet curly top in North America; however, sugar beet is more resistant to infection and much less severely injured.

The disease was transmitted by grafts but not by juice inoculation.

The vector, *Agallia albidula*, has a wide range of host plants. It breeds well on potato, common purslane, pigweed, jimsonweed, China-aster, lettuce, common nightshade, spinach, and spiny bur. The last-named probably is one of the important breeding plants in nature and one of the important sources of virus for crop plants. Apparently *A. albidula* cannot breed on tomato, tobacco, or sugar beet.

Evidence from infection from short successive transfers of individual leafhoppers on seedling plants indicates that the virus may be exhausted from certain insects but only after considerable periods.

Leafhoppers infected healthy seedling plants 24 to 48 hours after feeding on diseased plants, indicating that the minimum incubation period of the virus in the leafhopper was 24 hours or more. Viruliferous leafhoppers vary considerably in their ability to transmit the virus. In some instances they retained virus for 42 to 85 days when feeding on plants found immune from the disease. Progeny of these leafhoppers reared on the immune plants, however, did not transmit the disease, indicating that the causal virus is not transmitted through the egg of the vector.

The virus was precipitated from the juice of spinach in 50-percent alcohol and recovered by nonviruliferous leafhoppers that fed on a suspension of the alcoholic precipitate.

Because of the similarity in host range and in symptoms on various hosts it is thought that this virus is related to the sugar-beet curly top virus of the United States and to the curly top virus of Argentina; tentatively, at least, it should be classified as a variety of the curly top virus complex of the Western Hemisphere. In the system of classification and nomenclature presented by Holmes (*6*), it is suggested that the virus be designated *Ruga verrucosans* Carsner and Bennett var. *brasiliensis* n. var.

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SOME MOLDS ON WOOD FAVORED BY CERTAIN TOXICANTS[1]

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INTRODUCTION

During the past 6 years extensive field tests have been conducted in the Southern States on the chemical control of fungi on pine lumber during the air-seasoning period. Although these tests were primarily for the control of stain and decay fungi, observations were also made on mold occurrence. Under most commercial conditions molds do not cause degrade in seasoning lumber; but under some circumstances, and particularly with wood for such uses as food containers, molds may become a serious problem. In this paper are presented field observations on molds favored by certain chemical treatments of pine lumber and data from laboratory tests made to determine why certain molds are favored by some fungicides.

There are numerous reports in the literature showing that toxic chemicals are tolerated by certain fungi, particularly the molds. Arsenic compounds, for example, are tolerated by a number of fungi (*5*, *9*);[3] organic mercury salts by *Penicillium* spp. (*3*, *7*); methyl salicylate by some strains of *Fomes igniarius* Kickx (*10*); creosote and coal tar by *Hormodendrum resinae* Lindau (*2*); fluorides, sodium bisulfate, formaldehyde, and arsenious oxide by *Trichoderma* and *Gliocladium* (*7*); copper sulfate (*4*) and copper naphthenate (*1*) by *Aspergillus niger* v. Tiegh.; and others.

FIELD TESTS

The field observations were all made on wood in small-scale stain-control tests. For these tests 1- by 2- by 14-inch pieces of fresh green pine sapwood were dipped for about 10 seconds in aqueous solutions of fungicides. The wood was then piled in miniature air-seasoning piles and covered to reduce drying. Mold development, together with stain and decay prevalence, was determined after 4 to 6 weeks' exposure to natural infections. The chemicals that favored the development of molds are listed in table 1.

The molds observed were generally outstandingly more prevalent and luxuriant on wood treated with the chemicals for which they are listed than on the untreated controls. Under these severe test condi-

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[2] In cooperation with Southern Forest Experiment Station, U. S. Forest Service.

[3] Italic numbers in parentheses refer to Literature Cited, p. 703.

TABLE 1.—*Chemicals that favor the development of specific molds on treated green pine sapwood*

Chemical [1]	Tests	Concentration of treating solution	Fungus favored
	Number	*Percent*	
Borax	7	1.4 to 3.4	*Alternaria*.[2]
Ethyl mercuric phosphate	40	0.001 to 0.015	*Penicillium cyclopium* Westling.
Phenyl mercuric acetate	4	0.001 to 0.015	Do.
Cupric silicofluoride	[3] 1	1.2 and 2.4	*Trichoderma viride* Fr.
Magnesium silicofluoride	[3] 1	1.7 and 2.9	Do.
Sodium silicofluoride	[3] 1	1.9 and 2.9	Do.
Zinc silicofluoride	[3] 1	0.7	Do.
Tartar emetic	1	0.5	Do.
Urea [4]	1	4.3 and 8.6	*Trichoderma* and *Penicillium*.

[1] Phenyl mercuric acetate of c. p. grade; all other chemicals of technical grade. Ethyl mercuric phosphate in a proprietary containing 6.25 percent mercurial in borax and talc.

[2] Associated with the smoky-gray mold mentioned by Scheffer and Lindgren (*8*) on borax-treated pine. Further isolations would be necessary to establish that *Alternaria* is the only fungus concerned.

[3] The 4 silicofluorides were tested in 3 separate tests even though each was tested but once.

[4] Not favorable to molds at 50-percent concentration as used for chemical seasoning.

tions the fungi listed in table 1 were usually abundant on the treated wood, and molds other than these were usually inconspicuous or absent. On untreated controls in the same test piles *Alternaria*, *Penicillium*, *Trichoderma*, and other molds occurred to some extent; but usually the total mold growth was markedly less than on the treated wood. *Penicillium* and *Trichoderma* were observed on wood treated with a number of other chemicals, but the amount was not appreciably greater than that on the untreated controls.

The development of *Penicillium* on pine wood treated with ethyl mercuric phosphate is of particular interest. It has been found to be favored by both ethyl mercuric phosphate and ethyl mercuric chloride (*7*). Some strains of *Penicillium* sp. also have been reported as being highly resistant to other organic mercurials (*3*). In the present studies the extent and luxuriance of *Penicillium* were apparently increased by treatment of pine wood with 0.001 percent of ethyl mercuric phosphate and the increase continued with additional concentrations of the mercurial in the treating solution up to 0.015 percent, the full strength ordinarily used in lumber dipping (table 2). Full-strength solutions were used in a total of 40 tests. In nearly all of these the molding was heavier than on the untreated; the average mold rating was 2.2, as against 0.2 for the untreated.

Ethyl mercuric phosphate is differentially adsorbed from commercial lumber-treating solutions (*12*) so that they become progressively weaker as wood is passed through. It was found that the amount of fungus stain increased with continued use of the solution. The amount of *Penicillium*, however, decreased as the solution weakened (table 3).

LABORATORY TESTS

While in the field tests specific fungi grew more luxuriantly on treated wood, the tests did not show whether this increased development was due to a direct effect of the toxicant on the molds. Therefore, laboratory tests were made to ascertain this point for ethyl mercuric phosphate and sodium fluoride.

The following fungi were used:

Penicillium cyclopium (Gulfport 177), isolated from pine lumber treated with 0.015 percent ethyl mercuric phosphate. This is appar-

TABLE 2.—*Development of Penicillium on green pine sapwood treated with various concentrations of ethyl mercuric phosphate* [1] [2]

Mercurial in treating solution (percent)	Area showing *Penicillium* [3]			*Penicillium* rating [3] [4]				
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8
	Percent	*Percent*	*Percent*					
0 (undipped)	0		0			+	+	0.4
0 (water-dipped)		+		+	+	+		+
0.001						0.6	0.4	+
0.002	13				1.3	.9	.7	.1
0.003					2.0			
0.004	28	21	17	2.0		1.7	1.2	.1
0.005					2.4			
0.006					3.0			
0.008					3.2			.5
0.011					3.5			
0.015	84	79	64	3.0	3.7	3.7	2.4	1.8

[1] Mercurial in a proprietary containing 6.25 percent mercurial in borax and talc.
[2] This table includes all the tests enumerated in table 1 for which there were concentrations below 0.015 percent.
[3] + indicates a trace of growth.
[4] 0 (none), 1, 2, 3, 4, and 5 (very heavy).

TABLE 3.—*Development of Penicillium on green pine sapwood serially dipped in solutions of ethyl mercuric phosphate* [1] *until 80 or 90 percent of the volume of the original solution was consumed*

Volume of treating solution consumed (percent)	*Penicillium* rating [2]		Volume of treating solution consumed (percent)	*Penicillium* rating [2]	
	Test A [3]	Test B [4]		Test A [3]	Test B [4]
0–10	2.0	4.2	61–70	0.8	2.4
11–20	2.0	3.6	71–80	.6	1.5
21–30	1.8	3.3	81–90		1.5
31–40	1.7	3.0	Undipped		.2
41–50	1.3	2.5	Water-dipped	(5)	
51–60	.8	2.3			

[1] Mercurial in a proprietary containing 6.25 percent mercurial in borax and talc.
[2] 0 (none), 1, 2, 3, 4, and 5 (very heavy).
[3] Original solution was 0.5 gallon of 0.008 percent ethyl mercuric phosphate. Average number of test pieces per volume-reduction category was 27.
[4] Original solution was 1 gallon of 0.015 percent ethyl mercuric phosphate. Average number of test pieces per volume-reduction category was 44.
[5] Trace.

ently the same fungus listed by Scheffer and Lindgren (7) as *P. expansum*. The fungus mentioned by them as mercury tolerant was identified by Charles Thom as "*P. expansum* or some nearly related strain of this very common group." Gulfport 177, however, was identified at the Northern Regional Research Laboratory as the very closely related species *P. cyclopium*.

Trichoderma viride (Gulfport 178), isolated from pine lumber treated with 5-percent sodium fluoride.

Ceratostomella pilifera (Fr.) Wint. (Gulfport 146), isolated from pine lumber.

The sodium fluoride used was of a technical grade. The mercurial was in a proprietary containing 6.25 percent ethyl mercuric phosphate in borax and talc. Tap water was used in preparing the solutions.

For the first tests green pine sapwood was dipped for 5 seconds in aqueous solutions and immediately placed in moist chambers. These were allowed to stand for 24 hours so that the free toxic solution on the surface of the wood would be dissipated before their inoculation with 5-mm. disks of the mold colonies on malt agar. Incubation was

at room temperature. One block of wood (½ by 1 by 2 inches) dipped in each concentration was placed in each chamber. The fluoride and the mercurial were tested at different times. Five to eight replications were made for each category. The wood used was longleaf pine (*Pinus palustris* Mill.) and loblolly pine (*P. taeda* L.) sawed from the log about 2 days previously. Although bright at the time the tests were started, the wood was undoubtedly infected with a number of stain and mold fungi and other organisms. Half of the blocks were sterilized in an autoclave for about 20 minutes at 8 to 15 pounds' pressure to kill any organisms present; the other half were left unsterilized so that the inoculated mold was in competition with the microflora present in the wood at the time of collection. A few sterilized and unsterilized pieces were left untreated as controls, and a few pieces were inoculated with *Ceratostomella pilifera*, the most common stain fungus on green pine. The blocks for 0 concentration were dipped in water.

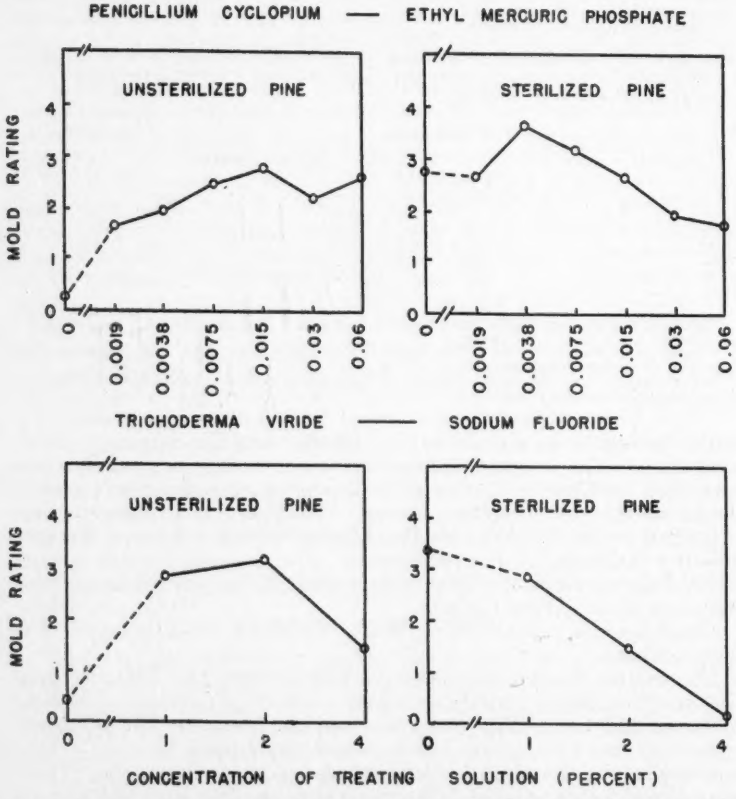

FIGURE 1.—Relative amount of mold on heat-sterilized or unsterilized pine sapwood treated with ethyl mercuric phosphate or sodium fluoride and inoculated with pure cultures of *Penicillium cyclopium* or *Trichoderma viride*, respectively.

After 2 weeks' incubation the mold growth on the blocks was rated on an arbitrary scale of 0 (none) to 5 (very heavy) based on area molded and luxuriance of the mold. Stain was recorded as percentage of area stained. The data are given in tables 4 and 5 and figure 1.

TABLE 4.—*Development of Penicillium cyclopium and Ceratostomella pilifera on unsterilized and heat-sterilized pine sapwood dipped in solutions containing various concentrations of ethyl mercuric phosphate* [1]

Mercurial in treating solution (percent)	Unsterilized wood [2]		Sterilized wood	
	Penicillium rating [3]	Area stained [4]	*Penicillium* rating [3]	Area stained [4]
		Percent		*Percent*
0	0.2±0.0	54	2.7±0.38	5
0.001875	1.6± .23	----	2.6± .40	----
0.00375	1.9± .37	4	3.6± .36	0
0.0075	2.4± .37	----	3.1± .26	----
0.015	2.6± .25	3	2.6± .42	0
0.03	2.1± .49	----	1.9± .39	----
0.06	2.5± .62	----	1.7± .33	----

[1] Mercurial in a proprietary containing 6.25 percent mercurial in borax and talc.
[2] On unsterilized, untreated, uninoculated blocks, 49 percent of the area stained and a trace of mold occurred. Sterilized, untreated, uninoculated blocks remained sterile.
[3] 0 (none), 1, 2, 3, 4, and 5 (very heavy).
[4] Mold and stain data from separate sets of wood blocks; all stain presumed to be due to *Ceratostomella pilifera*.

TABLE 5.—*Development of Trichoderma viride and Ceratostomella pilifera on unsterilized and heat-sterilized pine sapwood dipped in solutions containing various concentrations of sodium fluoride*

Fluoride in treating solution (percent)	Unsterilized wood [1]		Sterilized wood	
	Trichoderma rating [2]	Area stained [3]	*Trichoderma* rating [2]	Area stained [3]
		Percent		*Percent*
0	0.4±0.12	67	3.3±0.40	100
1	2.8± .49	20	2.8± .34	40
2	3.1± .24	20	1.4± .30	0
4	1.4± .44	0	.1± .01	0

[1] On unsterilized, untreated, uninoculated blocks 15 percent of area stained and *Trichoderma* got a rating of 1.0. Of the sterilized, untreated, uninoculated blocks 1 of 4 showed stain.
[2] 0 (none), 1, 2, 3, 4, and 5 (very heavy).
[3] Mold and stain data from separate sets of wood blocks; all stain presumed to be due to *Ceratostomella pilifera*.

There was a significant increase in the amount of *Penicillium* on the unsterilized wood treated with ethyl mercuric phosphate as compared with that on the untreated. The increased growth on unsterilized wood with each increase in concentration of the mercurial in the treating solution (fig. 1) is of low apparent significance because of the large standard errors (table 4). The rather large standard errors shown in this table (also in table 5) are partly due to the difficulty of standardizing the arbitrary ratings used and to possible differences between the extraneous organisms present on the blocks in the same moist chamber. However, in six of the seven replications there was a definite upward trend as the concentrations increased from 0 to 0.0075 percent and in four of these six cases up to 0.015 percent. Even at 0.06 percent, four times that needed to give control of stain and of most mold fungi, there was much more *Peni-*

cillium than on the untreated wood. Therefore, these data substantiate the field tests, showing an increase in molding with increased concentration and a marked increase over that on the untreated wood.

In the sterilized series the increase in growth of *Penicillium* when the concentration of the treating solution was increased from 0 to 0.00375 percent cannot be considered significant because growth at the lower three concentrations was unduly influenced by two of the eight replications. The indication is that the growth of *Penicillium* in the sterilized series decreased gradually as the concentration of the mercurial increased. The gradual slope of the curve between 0.00375- and 0.03-percent concentrations further indicates a high degree of tolerance to the mercurial. Sterilizing the wood increased the level of molding on the untreated wood as much as did the fungicidal treatment.

In the unsterilized series (table 5) *Trichoderma* grew significantly more on wood treated with sodium fluoride than on untreated wood. There was no significant difference between the growth at 1-percent concentration and that at 2-percent, but the 4-percent concentration appeared too strong for the best growth of the fungus. In the sterilized series there was a large increase in mold on the untreated as compared with the unsterilized, untreated series, similar to that in the case of *Penicillium*. On the sterilized wood the curve of growth is a typical toxicity curve with no indication of any increased fungal growth due to the fluoride.

The data show that on wood ethyl mercuric phosphate was less toxic to *Penicillium* than to the stainer *Ceratostomella pilifera*. Also sodium fluoride was less toxic to *Trichoderma* than to *C. pilifera*. In one test on malt agar, *C. pilifera* showed only a trace of growth at concentrations of 0.00014- and 0.00025-percent ethyl mercuric phosphate whereas *Penicillium* grew well at 0.0005 percent, the highest concentration tested.

To examine the possibility of stimulation by the mercurial at very low concentration, toxicity trials were made on a liquid medium in which weights of colonies were used to give a more exact measure of the growth of *Penicillium* and in which no competing organisms would complicate the interpretations.

Each concentration (table 6) was prepared by using 968 cc. of Richards' solution plus enough solution of ethyl mercuric phosphate and enough water to bring the total volume up to 1,000 cc. Both solutions were prepared in stock batches to minimize extraneous variations among the different concentrations. The nutrient solution plus the needed extra water was sterilized for 30 minutes at 10 to 15 pounds' pressure. When it was cool the mercurial solution was added with a burette, the flasks were shaken, and 200 cc. of each concentration was poured into each of two 500-cc. flasks. These were inoculated with the spores of *Penicillium cyclopium* adhering to an inoculating needle touched to an actively growing colony. The inoculated flasks were incubated at room temperature ($25.6° \pm 2.8°$ C.) for 7 days. The colonies were then removed, placed on weighed filter paper, dried at 50°, and weighed. The growth weights are recorded in table 6.

The data from test 1 indicate increased growth at very low concentrations of the mercurial, but the effect could not be accurately appraised for lack of gradations in the lowest part of the concentration range. A second test was therefore made in which most of the concentrations were lower than the lowest used in test 1. From the results of test 2 it appears that there is a definite, even though slight, growth increase associated with low concentrations of the mercurial. The general growth rate was lower than in the first test, however, because of cold weather during the first 4 days; during the last 3 days the incubator was electrically heated to about 25° C.

TABLE 6.—*Growth of Penicillium cyclopium in Richards' solution containing various concentrations of ethyl mercuric phosphate* [1]

Test and concentration of ethyl mercuric phosphate (p. p. m.)	Weight of colony		
	Flask 1	Flask 2	Average
Test 1:	*Grams*	*Grams*	*Grams*
0	1.99	1.95	1.97
0.16	2.10	2.18	2.14
0.31	1.92	2.06	1.99
0.63	1.58	1.58	1.58
1.25	.83	.22	.53
2.5	.09	.12	.11
5	.04	.04	.04
Test 2:			
0	1.33	1.28	1.31
0.0006	1.59	1.27	1.43
0.006	1.66	1.63	1.65
0.063	1.68	1.82	1.75
0.63	1.39	1.41	1.40

[1] Mercurial in a proprietary containing 6.25 percent ethyl mercuric phosphate in borax and talc.

The concentrations of mercurial in the liquid-medium tests are not directly comparable with those in the wood tests. On the treated wood the actual mercury concentrations may be greatly reduced because of the high moisture content of the wood, loss through volatilization of the mercurial, and adsorption by the wood fibers.

DISCUSSION AND CONCLUSIONS

Observations on field tests for chemical control of fungi on green lumber during the air-seasoning period showed that several molds tolerated specific chemicals in concentrations that were effective in controlling most of the wood-inhabiting fungi. The tolerance of *Alternaria* to borax militates against the use of borax on pine except in mixtures with chlorophenates. *Alternaria* was commonly isolated from pine but seldom from hardwoods (*11*), to which borax alone usually provides satisfactory protection. The high tolerance of *Penicillium* to organic mercurials permits appreciable molding of lumber in commercial operations mainly in the Pacific Northwest and Scandinavia. Apparently *P. cyclopium* develops more luxuriantly in cool climates. In the tests herein reported the heaviest molding usually occurred during the winter. *Penicillium* will tolerate ethyl mercuric phosphate on wood treated with solutions at least four times that ordinarily used for protection against stain and decay fungi. The luxuriant growth of *Trichoderma* on wood treated with several chemicals, particularly the fluoride salts, makes these unsuited for use in

protecting green lumber during air seasoning, but some have found favor in more permanent wood preservation.

Laboratory tests with *Trichoderma* on fluoride-treated wood and both laboratory and field tests involving *Penicillium* on mercurial-treated wood indicate that heavy mold development on pine sapwood treated with these chemicals is due not only to the high tolerance of these molds to the specific chemicals but also to the fact that very low concentrations of them actually favor development of the respective molds. Similar favorable effect by minute amounts of fungicidal chemicals has been reported for several toxicants and wood-decaying fungi (*5*, *6*).

Interpretation of the observed favorable effects of chemicals is not always easy. Sterilizing the wood with heat had the same favorable effect on the mercurial- and fluoride-tolerant molds as did the use of the respective fungicides. Some of this increased molding on heat-treated wood may have been due directly to the heat rendering the wood more susceptible to mold attack. However, this also suggests strongly that the increased growth on chemically treated wood is due, at least in part, to the elimination by the fungicides of competitive micro-organisms. Even though this competitor-elimination hypothesis seems the most logical, the data do not rule out other possibilities. None of the alternate possibilities that come to mind seem likely to exert sufficient influence to affect materially the observed influences of chemical treatments in favoring molds.

The luxuriance and extent of *Penicillium* development on unsterilized green pine increased with the concentration of mercury in the treating solution. This may be explained reasonably on the basis that competing organisms vary in their tolerance to mercury. As the mercury concentration increases more species would be progressively screened out, and this progressive decrease in numbers of antagonistic organisms would permit a progressive increase in the growth of *Penicillium*.

The tests in which the *Penicillium* was grown on Richards' solution indicate that there is something more in the effect of ethyl mercuric phosphate than is considered in the foregoing hypothesis. In the entire absence of competing organisms and of wood, increase in fungal growth through the range of concentrations from about 0.0006 to 0.06 p. p. m. was too consistent to be ignored. However, the increase was scarcely large enough to explain all of the effects observed on treated wood. To classify positively this effect of the chemical on growth in nutrient solution as stimulation would require broadening the term to include purely nutritional effects, since there is no certainty that the chemicals added did not supply some nutrient material that Richards' solution lacked. All the chemicals used in preparing the nutrient solution were of c. p. grade; therefore they may have lacked trace elements not supplied by the tap water used. The mercurial, on the other hand, was in a proprietary mixture containing, in addition to the mercurial, borax, talc, and materials to give a warning color and odor. Some of these were presumably of technical grade and possibly contained impurities.

SUMMARY

Field tests showed that treatment of green pine sapwood with some fungicides favored heavy development of certain molds. Borax favored the development of *Alternaria*, silicofluorides and tartar emetic *Trichoderma viride*, and organic mercurials *Penicillium cyclopium*. Urea favored both *Trichoderma* and *Penicillium*.

The extent and luxuriance of *Penicillium cyclopium* on wood treated with ethyl mercuric phosphate increased as the concentration of the treating solution was increased up to that used commercially for stain control.

Laboratory tests with *Trichoderma viride* on fluoride-treated and *Penicillium cyclopium* on mercurial-treated wood suggested that the luxuriant growth of these molds on treated wood was due in part to elimination of competing organisms by the fungicides. Heat sterilization of nonchemically treated wood increased the level of molding as much as did fungicidal treatment. Tests further showed that these molds are highly tolerant to the respective fungicides. In the case of *P. cyclopium* very low concentrations of the mercurial actually favored development even in pure culture.

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FALSE MOTES IN COTTON: THEIR ORIGIN, DESCRIPTION, AND VARIATIONS IN NUMBER[1]

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INTRODUCTION

Seed cotton contains various numbers of aborted seeds termed "motes" (*5*).[2] These structures range from small ones with very short fibers to larger ones with fibers practically as long as those on mature seeds (*5*, *7*). Motes have been described, and variations in their number in seed-cotton samples representing different varieties, environments, pickings, lock sizes (number of ovules, or potential seeds, per lock), and number of locks per boll have been observed (*1*, *2*, *5*, *7*, *8*, *9*).

At the base of certain locks, however, there occur very small motelike bodies which heretofore have not been recognized as being distinct from true motes. These structures are motelike in that they consist of small, hard, seedlike bodies to which short fibers (as compared with those on mature seeds) are attached. They differ from motes in that each is derived not from a normal ovule but from a small rudimentary structure seemingly incapable of maturing into a seed. These rudimentary structures are herein termed "basal bodies" and the motelike structures into which they develop are termed "false motes." This paper describes both the basal bodies and the false motes found in upland cotton (*Gossypium hirsutum* L.) and presents information regarding the extent to which the number of false motes found in different seed-cotton samples varied with variety, environment (location and season of growth), lock size, and number of locks per boll. No attempt is made herein to consider in detail possible explanations for these variations or for the nature of the basal bodies.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 717.

MATERIALS AND METHODS

Special plantings, made at Stoneville, Miss., in 1937, of the same 16 varieties included in the 1935–37 cooperative regional variety investigations of the Bureau of Plant Industry, Soils, and Agricultural Engineering (*6*) supplied the ovaries and the young and mature bolls used in studying the origin and characteristics of false motes.

The same plantings also supplied seed-cotton samples used in making some of the observations reported concerning the variation in number of false motes with variety, and with the number of ovules per lock and of locks per boll. These seed-cotton samples, which will be referred to as the "special-planting samples," were obtained as follows: For each variety 75 blooms were tagged approximately every other day throughout the blooming period. Each variety sample consisted of all the bolls matured from these blooms. In collecting the mature seed cotton, the burs were snapped and thus the locks were left intact.

Two sets of seed-cotton samples which were included in the 1935–37 cooperative regional variety investigations of the Bureau (*6*)[3] were also studied.

The data from 1 set were analyzed statistically to show the extent to which the production of false motes varied with variety and location and season of growth. This set represents what is known as the spinning test series (*4*) and includes 767 samples representing 2 series for each of 16 varieties grown at 8 locations in 3 successive years.[4] The varieties and the locations are listed in table 1. The samples consisted of 100 locks each and were selected from the 2-pound samples of seed cotton taken to represent the spinning test series. To make up the 100-lock samples, each 2-pound sample was divided into 4 lots and 25 locks were taken from each.

The other set of samples was used to show variation in false-mote formation with numbers of ovules per lock and of locks per boll. This set was composed of 768 samples and represents 4- and 5-lock bolls for 8 series of the same 16 varieties used in the spinning test series, but for 3 locations (Lubbock, Tex., Statesville, N. C., and Baton Rouge, La.) in 1937 only. Each sample studied consisted of 100 locks selected from the original 100-boll samples, an effort being made to have each boll represented by 1 lock. To do this, each 100-boll sample was divided into 4 or 5 lots and an even distribution of the locks of each boll among all the lots was attempted. Such a distribution, of course, could not be accomplished with certainty; for, though the locks comprising a boll could frequently be recognized as such, a part of every sample was composed of separate or even broken locks. To make up 1 sample of 100 locks for each 100-boll sample, it was usually necessary to add to 1 of the subsamples a few

[3] BARRE, H. W. NATURE AND SCOPE OF THE COOPERATIVE REGIONAL VARIETY STUDIES WITH COTTON. Paper presented before the Amer. Soc. Agron., New Orleans, La., Nov. 22–24, 1939. 5 pp. [U. S. Bur. Plant Indus. processed copy.]

[4] There were 767 samples rather than 768 ($16 \times 2 \times 8 \times 3$), for in 1 instance replicates were composited. To facilitate analysis and to have equal numbers of observations, the percentage figure for the composited sample was assigned to the 2 replicates. In view of the large number of observations and the great consistency in the data, this procedure seemed justified.

locks taken from the other 3 or 4 lots into which the original 100-boll sample had been divided.

The regional variety samples consisted of picked cotton. In such samples there is always the possibility that the basally located seeds and motes and even the false motes may have been left in the bur. It was attempted, however, in making up the 100-lock subsamples, to select only locks that appeared to be intact.

The examination of the seed cotton consisted of determining for every lock in a sample whether a false mote was present and the number of ovules represented (seeds and motes). The relative number of false motes in a sample is reported as the percentage of locks in which they were found to occur.

ORIGIN AND DESCRIPTION OF FALSE MOTES

Flowers examined on the day of blooming show many carpels each possessing a small body located between and just below the two basal ovules (fig. 1, *A*). These bodies do not appear to be in line with either placental ridge. They can be distinguished readily from the ovules by their smaller size and distinct location. They vary somewhat in size; but in the hundreds of ovaries that have been examined, no basal body which anywhere nearly approaches ovular size has been observed. They are not all of the same shape. The majority observed were small, rounded or elongated structures with no macroscopic evidence of chalazal or micropylar differentiation (fig. 1, *A*). Some, however, resembled ovules in general shape, and of these some possessed beaklike distortions of what might be considered the micropylar or the chalazal region (fig. 1, *B* and *C*).

Only rarely is there more than one basal body in a single carpel or is a similar body found at positions other than the base. In the course of these and other investigations several hundred ovaries have been examined; there have been found only two carpels in which two small bodies were situated below the two basal ovules and only one carpel in which a similar body was located in the middle of one of the rows of ovules.

No extensive microscopic study of the basal bodies has as yet been made. Sections through a few that were preserved on the day of anthesis show that at that time these particular ones consisted of an epidermis surrounding a mass of parenchymatous tissue into which there passes a vascular strand. There were no indications of the presence of an embryo sac (fig. 1, *D*).

Development of the basal bodies into false motes was not followed microscopically. Macroscopic observations on bolls of different ages from the day of flowering to maturity showed conclusively, however, that the small basal bodies found in carpels on the day of flowering ultimately develop into the small, basally located, motelike bodies found in mature bolls (fig. 1, *E*).

False motes vary in size (fig. 1, *F*). Such variation in the main, however, represents differences in length and in number of fibers, though the dried basal bodies may themselves differ slightly in size. Increases in fiber length are not necessarily associated with increases in fiber population, for occasionally false motes were found with

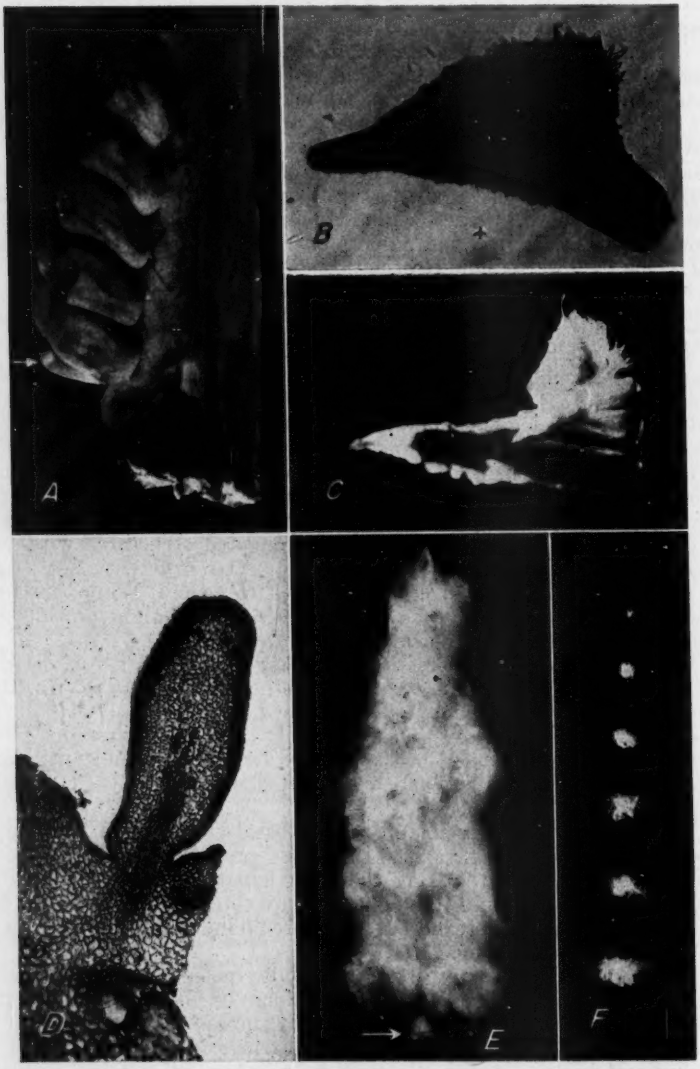

FIGURE 1.—A, Ovules and basal body of one carpel dissected from the rest of the ovary on the day of flowering; basal body indicated by arrow. × 12. *B*, Basal body (false mote) from a 2-day-old boll. × 50. *C*, False mote together with adjacent placental tissue, from a 3-day-old boll, showing fiber development and beak at the chalazal (?) end. × 12. *D*, Nearly median longitudinal section through a basal body, day of flowering. × 67. *E*, Mature lock of cotton; false mote indicated by arrow. × 3/5. *F*, False motes from mature locks, showing typical range in size found in one variety. × 1.

only a few but nevertheless comparatively long fibers. No attempt was made to obtain accurate measurements of fiber length. From examination of hundreds of these structures, however, it was concluded that only occasionally do the fibers exceed 1 cm. in length and in the great majority of cases the fiber length is much less.

There is considerable variation in size within any one variety (fig. 1, *F*). The possibility of some varietal differences in false-mote sizes was noted, but the differences observed did not seem to be sufficiently pronounced to warrant investigation at this time.

There is some indication that a few basal bodies fail to form fibers, for occasionally a small speck is found in dried burs at a position which corresponds to that of the basal body in a carpel. Observations indicate that this failure is rather rare and that differences in the number of false motes found in different lots of seed cotton would represent similar differences in the number of basal bodies that occurred in the corresponding carpels.

The fibers on false motes do not appear to be essentially different from those on seeds except that they are, of course, shorter. When mature, they normally possess a secondary wall of about the same thickness as that of a typical mature fiber on a seed, and the dried fibers are convoluted. Despite the fact that the structures which bear the fibers differ enormously in size, the cross-sectional areas (perimeters) of fibers of false motes appear to be about the same as those of seed fibers.

False motes not only are smaller than most of the motes derived from normal ovules but possess several seemingly distinguishing characteristics, which should serve to identify them when they are found in materials for which previous locations in locks would be unknown, such as in a ginned lint or products of the manufacturing processes. In general it may be stated that: (1) False motes have fewer fibers than true motes, though some small motes undoubtedly derived from normal ovules possess only a few short fibers; (2) the fibers of many false motes are longer in proportion to the mote body than those of true motes; and (3) false motes lack the slightly elongated delicate funiculus possessed by most small motes derived from ovules. Some structures, however, do not possess clear-cut false- or true-mote characteristics (*5*) and cannot be identified on the basis of general appearance alone.

Seed-cotton samples in which the locks have been left in the bur (snapped cotton) are the most satisfactory materials for studying the occurrence of false motes. In such material not only can location and general appearance be used for identification but the number of funicular scars, or points of ovular attachments, are of supplementary value in doubtful cases. If mature seeds occupy both basal positions, a false mote, if present, can be readily and positively identified (fig. 1, *E*). There is no structure with which it could be confused. If one or both of the basal positions of the lock are occupied by motes, however, a false mote, if present, might not be recognized. The false mote would be recognized with particular difficulty if one or both of the motes were very small or if the false mote were pushed to one side because of a difference in size of the two structures occupying the basal positions, a condition which occurs rather frequently.

Doubtful cases can be settled by comparing the number of funicular scars on the placental ridges with the number of seeds and motes (ovules) in the locks. The two values should be the same and the agreement or lack of agreement between them can be used to decide whether a questionable structure is derived from a basal body or an ovule.

Picked seed cotton which has been handled with care is nearly as satisfactory as snapped cotton. It is true that occasionally false motes are left in the bur when the locks are picked. In studying the snapped cotton it was observed, however, that when well-matured and fluffed locks are carefully removed from the bur the failure of false motes to be detached with the lock apparently does not happen frequently enough to affect the reliability of the samples.

VARIATIONS IN THE NUMBER OF FALSE MOTES IN SEED COTTONS

The discussion of variation in the number of false motes in different lots of seed cotton is based on data derived from a study of both picked and snapped cotton.

Varietal differences in the number of false motes formed are very striking (tables 1 to 3, figs. 2 and 3). For the 16 varieties represented in the 1935–37 cooperative regional variety investigations the average percentages of locks possessing false motes ranged from 4.7 percent for Mexican Big Boll to 24.2 percent for Deltapine 11. In general, the relative differences among the varieties were about the same from location to location and from year to year (tables 1 and 3, fig. 2). In spite of the consistency in behavior a highly significant tendency for varieties to exhibit different responses to environment was established (table 3).

TABLE 1.—*Locks with false motes in 100-lock samples for 2 series for each of 16 varieties of cotton grown at 8 locations for 3 successive years* [1]

Variety	Locks with false motes at—								Mean [2]
	Florence, S. C.[3]	Stoneville, Miss.[3]	Marianna, Ark.		Baton Rouge, La.[3]	Stillwater, Okla.[3]	College Station, Tex.[3]	Lubbock, Tex.[3]	
			Upland [3]	Delta [3]					
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Acala (Rogers)	24.5	19.3	21.0	12.2	19.0	20.3	19.0	26.2	20.2
Arkansas 17	14.0	12.7	11.7	11.0	8.3	14.7	11.5	14.3	12.3
Cleveland (Wannamaker)	14.5	10.7	16.5	11.0	12.2	15.0	13.7	17.0	13.8
Cook 912	9.5	7.5	8.3	4.3	5.8	7.2	5.0	9.7	7.2
Delfos (Missdel) 4	24.3	16.2	22.0	13.7	16.5	15.2	12.3	18.5	17.3
Deltapine 11	25.0	22.3	28.2	17.8	22.3	25.0	24.0	28.7	24.2
Dixie Triumph 759	23.3	13.2	22.5	13.7	21.2	18.2	21.3	20.0	19.2
Farm Relief 2	33.3	23.2	19.3	16.8	22.8	20.2	19.0	22.0	22.1
Half and Half	20.3	14.7	19.3	10.0	10.5	21.0	14.8	18.2	16.1
Mexican Big Boll	4.5	4.8	5.8	3.3	2.8	4.5	3.3	8.2	4.7
Qualla	14.3	8.7	11.8	6.2	10.5	5.5	8.0	6.8	9.0
Rowden 40–2088	26.0	23.5	26.7	16.0	21.8	24.2	23.7	24.2	23.2
Startex 619	21.0	13.5	16.5	11.0	15.8	15.5	13.2	18.5	15.6
Stoneville 5	33.8	16.2	25.5	16.3	22.0	26.3	20.0	29.0	23.6
Triumph (Oklahoma) 44	18.7	14.8	16.7	10.0	15.3	14.0	11.8	15.0	14.5
Wilds 5	21.7	15.7	16.3	11.8	13.8	11.2	9.7	17.8	14.8
Mean [4]	20.6	14.8	18.0	11.6	15.0	16.1	14.4	18.4	

[1] Least significant difference between—	*5-percent level*	*1-percent level*
Variety means	1.7	2.2
Location means	1.2	1.6

[2] Values based on 6 observations.
[3] Values based on 48 observations.
[4] Values based on 96 observations.

TABLE 2.—*Locks with false motes in samples for each of 16 varieties of cotton representing 2 plantings and 2 types of harvesting, Stoneville, Miss., 1937*

Variety	Locks with false motes from—		Variety	Locks with false motes from—	
	Snapped cotton (special planting)[1]	Picked cotton (regional variety planting)[2]		Snapped cotton (special planting)[1]	Picked cotton (regional variety planting)[2]
	Percent	*Percent*		*Percent*	*Percent*
Acala (Rogers)	20.7	32.5	Half and Half	18.6	22.5
Arkansas 17	12.8	22.0	Mexican Big Boll	2.6	8.5
Cleveland (Wannamaker)	19.2	20.0	Qualla	7.0	17.0
Cook 912	3.3	10.5	Rowden 40-2088	30.6	41.5
Delfos (Missdel) 4	27.3	23.5	Startex 619	16.7	17.5
Deltapine 11	29.9	32.5	Stoneville 5	27.1	21.5
Dixie Triumph 759	21.0	24.0	Triumph (Oklahoma) 44	14.4	23.0
Farm Relief 2	25.8	33.0	Wilds 5	19.2	23.5

[1] Values based on at least 1,400 locks.

[2] Values based on 2 samples of 100 locks each.

TABLE 3.—*Analysis of variance of the percentage of locks with false motes in 100-lock samples for 2 series for each of 16 varieties of cotton grown at 8 locations for 3 successive years*

Source of variation	Degrees of freedom[1]	Mean square	Source of variation	Degrees of freedom[1]	Mean square
Varieties	15	1,670.9**	Years × blocks within locations	16	20.3
Locations	7	751.9**	Variety × years × locations	210	28.9**
Years	2	2,608.2**	Error	359	17.5
Blocks within locations	8	122.3**			
Varieties × years	30	40.3**	Total	766	83.6
Varieties × locations	105	35.2**			
Years × locations	14	681.3**			

**Significant at the 1-percent level.

[1] In 1 instance replicates were composited, reducing the degrees of freedom from the expected 767 to 766. To facilitate analysis and make equal numbers of observations the percentage figure for the composited sample was assigned to the 2 replications.

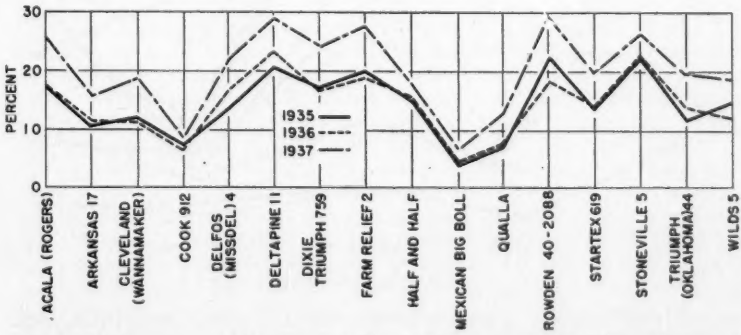

FIGURE 2.—Average percentages of locks with false motes for 16 varieties of cotton grown in 1935, 1936, and 1937. Each plotted point is the mean of 16 observations, 2 series at each of 8 locations. Each observation is based on a 100-lock sample.

The varietal differences shown by the Stoneville 1937 picked-cotton, regional variety samples were shown with about equal consistency, also, by the concurrently grown, snapped-cotton, special-planting samples (table 2). Contrary to what might be expected, however, the percentages of locks with false motes were, in general, higher for the picked than for the snapped cotton. The difference may possibly be explained by the seasonal trend in false-mote formation, together with possible differences in the relative number of bolls from the different portions of the blooming periods in the two sets of samples. In 1937 at Stoneville, Miss., as shown by the snapped-cotton samples, the bolls from early and midseason blooms possessed more false motes than those from late blooms (fig. 3). The period of greatest false-mote production thus undoubtedly corresponds to or at least is included in the period of greatest boll production. The particular picked-cotton samples being considered here represent practically entire crops (spinning test series). The snapped-cotton samples, it will be recalled, were made of bolls developed from flowers tagged on approximately alternate days throughout the blooming period. Be-

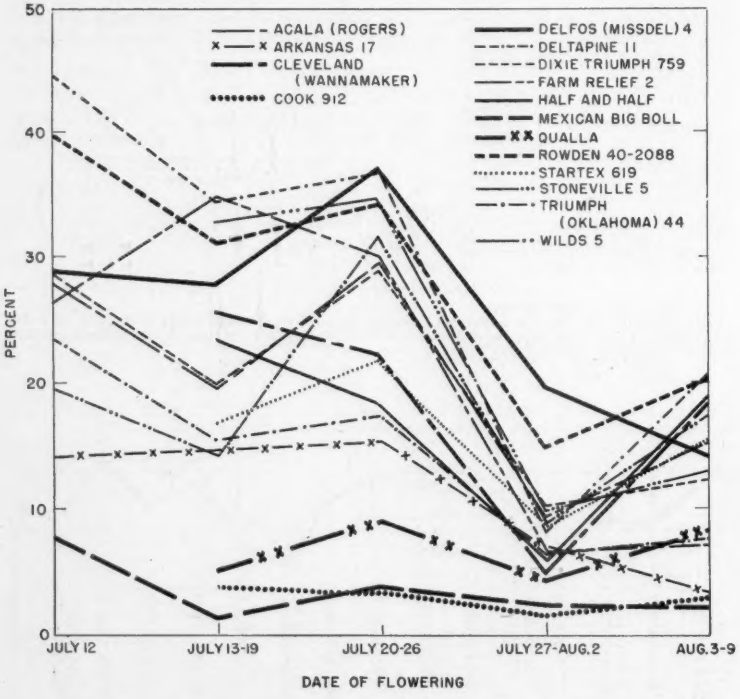

FIGURE 3.—Percentages of locks with false motes in bolls developed from blossoms tagged approximately every other day throughout the blooming period, grouped by week of flowering, for 16 varieties of cotton grown at Stoneville, Miss., in 1937. Percentage figure for each plotted point is based on a 100+-lock sample.

cause of this difference in composition, it is very likely that the picked-cotton samples contained relatively more seed cotton representing the period of greatest boll production and consequently more false motes than did the snapped-cotton samples.

The importance of the influence of environmental factors on the development of false motes is shown by the highly significant differences in the number of false motes in seed-cotton samples representing different locations and seasons of growth (table 3, fig. 4). On the whole, climatic factors, as represented by differences in seasons, appeared to have much more influence on the number of false motes developed than did environmental factors associated only with differences in place of growth and even more influence than did varietal factors (table 3). Though locational factors taken as a whole did appear to have a pronounced influence on the relative number of false motes formed (table 3), there was no significant tendency for location rank to remain the same from year to year (fig. 4). This is to be expected since seasonal (climatic) factors, which apparently have

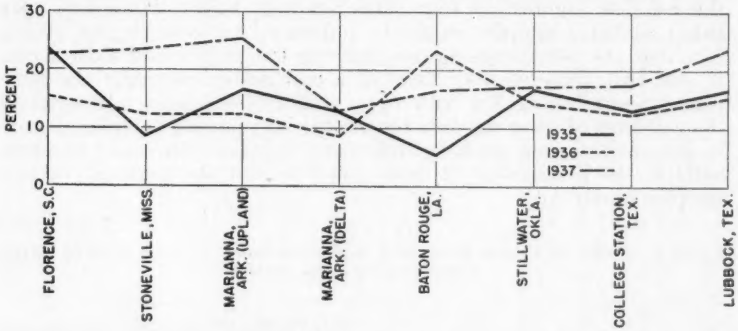

FIGURE 4.—Average percentages of locks with false motes for cotton grown at 8 locations in 1935, 1936, and 1937. Each plotted point is the mean of 32 observations, 2 series for each of 16 varieties. Each observation is based on a 100-lock sample.

such a pronounced effect on the formation of false motes, would not be expected to be alike at all locations in any one year or even alike at any one location every year.

The highly significant differences that were found between blocks at the same location (table 3) are probably another indication of the importance of environmental influences on false-mote formation.

The tendency for early and midseason blooms to produce bolls with more false motes than late blooms could not be satisfactorily related to the climatic conditions prevailing during the blooming periods (fig. 3) (*5*). This trend in false-mote formation might possibly be related to the physiological condition of the plants during early, middle, and late periods of bud formation, but more data would be needed to establish this with certainty. It is very probable that the trend shown in this one year would not be the same in other years.

The production of false motes varied also with the numbers of ovules per lock and of locks per boll. Differences between four- and

five-lock bolls were the more striking, false motes being produced in relatively much greater numbers in five- than in four-lock bolls. This tendency was shown both by the regional variety samples and by the special-planting samples (table 4). Since the two sets of samples differed in manner of selection, in size and uniformity, and consequently in sources of error, data for both sets are presented. The regional variety samples, it will be recalled, were of uniform size (100 locks each). They were made up of picked cotton, however, and the possibility that some false motes were not removed must be taken into consideration. Nevertheless, the data derived from these samples were very consistent with regard to varietal, locational, and boll-type differences in the number of false motes produced. In fact, they were not analyzed statistically since they show so strikingly and consistently that five-lock bolls produce false motes in relatively greater numbers than do four-lock bolls.

The special-planting variety samples, on the other hand, were not uniform in size, being made up of all bolls that matured from tagged blooms. They differed not only in the total number of bolls but in the relative number of four- and five-lock bolls. Moreover, data based on these samples would be influenced to some degree by the fact that the percentage figures showing the presence of false motes in one, two, three, or four locks of a boll do not represent the same percentage relations for both four- and five-lock bolls. In spite of these sources of error the data for the special-planting samples showed in general the same striking differences between four- and five-lock bolls in the production of false motes as did the regional variety samples (table 4).

TABLE 4.—*Locks with false motes in 4- and 5-lock bolls of 16 varieties of cotton grown at 4 locations, 1937*

Variety	Locks with false motes in bolls at—							
	Lubbock, Tex.[1]		Statesville, N. C.[1]		Baton Rouge, La.[1]		Stoneville, Miss.[2]	
	4-lock	5-lock	4-lock	5-lock	4-lock	5-lock	4-lock	5-lock
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Acala (Rogers)	12.7	32.4	22.5	35.9	9.4	18.1	7.8	23.6
Arkansas 17	10.9	24.9	17.0	29.9	6.9	13.0	9.4	16.9
Cleveland (Wannamaker)	17.1	25.9	17.2	24.9	9.0	14.2	18.4	19.5
Cook 912	4.5	10.2	4.4	14.5	3.5	5.6	2.0	3.9
Delfos (Missdel) 4	12.8	32.9	36.0	47.4	7.0	23.5	18.8	38.2
Deltapine 11	39.1	45.6	35.9	44.4	25.2	21.9	26.8	34.1
Dixie Triumph 759	20.0	28.2	25.1	29.4	17.8	17.9	19.8	21.2
Farm Relief 2	19.2	40.1	35.5	42.2	22.9	30.9	20.8	29.5
Half and Half	21.6	40.2	18.4	36.2	6.6	14.0	17.0	19.5
Mexican Big Boll	1.1	3.6	2.0	7.6	2.0	3.2	1.7	2.6
Qualla	4.6	9.1	5.9	19.0	9.0	13.9	2.1	8.1
Rowden 40-2088	27.9	38.5	33.8	44.4	27.1	32.1	24.2	34.4
Startex 619	16.6	28.2	23.4	39.5	10.8	21.4	7.6	18.7
Stoneville 5	15.4	38.0	27.9	45.4	8.2	26.0	19.1	30.8
Triumph (Oklahoma) 44	12.4	24.9	17.6	35.5	9.4	14.1	11.1	15.1
Wilds 5	14.9	24.0	35.5	45.0	14.6	18.4	14.6	22.3
Mean	15.7	27.9	22.4	33.8	11.8	18.0	13.8	21.2

[1] Regional variety samples of picked cotton; values based on 8 observations, each based on a 100-lock sample.

[2] Special-planting samples of snapped cotton; values based on samples of not less than 270 locks, the majority containing 500–1,000 locks each.

For 8 of the 16 varieties grown at 3 locations in 1937 and for the 16 varieties of the special-planting samples the percentage of locks that possessed false motes were calculated for 7-, 8-, 9-, and 10-ovule locks (table 5). The data were not analyzed statistically since the number of locks in the different lock-size samples was not uniform. The results are not entirely consistent, but in general and for most varieties there is a fairly marked tendency for the percentage of locks with false motes to decrease as the number of ovules per lock increases (table 5). It is true that certain varieties failed to show this trend, and some showed it to a much more pronounced degree than others. There were no apparent explanations for these differences in behavior.

TABLE 5.—*Locks with false motes in 7-, 8-, 9-, and 10-ovule lock-size classes for varieties of cotton grown at 4 locations, 1937* [1]

Location	Variety	Locks with false motes in indicated lock-size class			
		7-ovule	8-ovule	9-ovule	10-ovule
		Percent	*Percent*	*Percent*	*Percent*
Statesville, N. C.[2]	Delfos (Missdel) 4	47.4	41.2	39.3	----
	Deltapine 11	55.1	42.9	32.2	----
	Farm Relief 2	40.9	41.1	33.2	----
	Half and Half	----	25.4	28.3	23.3
	Rowden 40–2088	[3] 45.3	41.5	37.9	[3] 31.6
	Startex 619	----	37.0	30.5	21.4
	Stoneville 5	50.4	37.9	32.1	----
	Wilds 5	44.2	41.6	40.0	----
Baton Rouge, La.[2]	Delfos (Missdel) 4	16.8	15.5	14.0	----
	Deltapine 11	27.6	28.5	20.4	6.1
	Farm Relief 2	29.5	27.7	23.0	----
	Half and Half	----	14.7	9.8	8.9
	Rowden 40–2088	33.1	35.6	29.6	----
	Startex 619	[3] 26.2	19.4	16.2	9.8
	Stoneville 5	24.4	20.9	13.2	----
	Wilds 5	16.2	18.0	18.0	[3] 4.3
Lubbock, Tex.[2]	Delfos (Missdel) 4	27.4	19.4	20.8	----
	Deltapine 11	47.4	41.5	34.1	----
	Farm Relief 2	36.2	30.2	22.2	----
	Half and Half	37.6	35.3	29.4	18.3
	Rowden 40–2088	34.3	34.7	32.5	25.2
	Startex 619	22.6	24.9	21.4	17.4
	Stoneville 5	33.9	22.3	[3] 25.2	----
	Wilds 5	20.9	18.8	20.6	----
Stoneville, Miss.[4]	Acala (Rogers)	27.6	19.8	15.7	----
	Arkansas 17	12.6	12.0	14.6	----
	Cleveland (Wannamaker)	24.4	18.4	15.8	----
	Cook 912	8.5	3.3	2.0	1.1
	Delfos (Missdel) 4	31.9	26.6	21.9	----
	Deltapine 11	34.8	30.7	19.1	----
	Dixie Triumph 759	17.8	22.5	24.8	----
	Farm Relief 2	23.6	26.0	26.7	----
	Half and Half	26.6	22.1	16.5	10.2
	Mexican Big Boll	5.1	2.4	7.2	----
	Qualla	----	8.6	7.7	5.1
	Rowden 40–2088	33.5	32.2	29.4	26.7
	Startex 619	----	19.1	16.6	12.0
	Stoneville 5	26.5	27.2	27.1	----
	Triumph (Oklahoma) 44	22.3	12.0	15.0	6.0
	Wilds 5	24.3	20.0	17.7	----

[1] Values based on at least 100-lock samples except as indicated.
[2] Regional variety samples of picked cotton.
[3] Value based on 50- to 99-lock sample.
[4] Special-planting samples of snapped cotton.

DISCUSSION

False motes may be a source of error in calculating the percentage figures for the number of motes in a particular seed-cotton sample (*5*). These percentages are based on the relation of the number of aborted seeds, or motes, present in a sample to the total number of ovules

represented (*1*, *2*, *5*, *7*, *9*). If the false motes are included with the true motes derived from ovules, the basal bodies that gave rise to the false motes would then be considered as ovules. Since basal bodies do not mature into seeds, percentage figures based on counts which included them would give an entirely inaccurate representation of the number of potential seeds that failed to mature (formed motes). It is fairly evident that Porter (*7*) included false motes in his mote counts, for he found an unusually large number of motes located at the base of the lock (*5*). Data representing the mote content of seed-cotton samples reported by different investigators must have been derived in a like manner to be comparable (*5*).

False motes are probably not such an important source of small imperfections in yarn and cloth as are the true motes developed from ovules (*3*). The dried basal body is too small to be broken up into fragments that persist in the yarn. Entire false motes have not been found or at least have not been recognized in appreciable numbers in either yarn or ginned lint, though in the latter they may have been overlooked because of their small size. They apparently do not cling very tenaciously to the lint, many of them being removed either during ginning or later during cleaning and spinning.

The early developmental history of the basal bodies has not yet been studied. That these bodies might be rudimentary ovules could be inferred from their location, behavior, and general relations, and from the fact that some are definitely ovular in general form. Their location close to normal ovules and the fact that they likewise produce fibers from their epidermal cells would indicate an ovular nature. A rather marked tendency within varieties for the percentage of locks with false motes (carpels with basal bodies) to decrease with an increase in the number of ovules per carpel might perhaps be another such indication (table 5).

Although false-mote formation was shown to vary significantly with variety and environment, consistent relations with varietal and environmentally induced variations in the amount of seed abortion (*5*) were not established.

What varietal and environmental factors are responsible for variations in the number of false motes formed, or rather for the occurrence of basal bodies, and how these factors operate cannot be answered from the information available. It would seem reasonably certain, however, that the factors concerned must have exerted their influence a long time before the day of anthesis, for on that particular day, as well as for several previous days, the distinctive nature of the basal body is well established.

SUMMARY

At the base of certain locks of cotton occur small motelike structures herein designated as "false motes." These structures are motelike in that each consists of a small body to which fibers are attached. They differ from motes in that they are not derived from ovules; each one develops from a small rudimentary body situated just below and between the two basal ovules and seemingly not in line with either placental ridge. These bodies are referred to herein as "basal bodies." They are distinctly different from ovules, being very much smaller and apparently incapable of seed formation.

False motes may differ very little from some very small true motes. Identification of false motes in intact locks of seed cotton is made chiefly on the basis of position, though appearance characteristics such as fiber length, density of fiber population, size of seedlike body, and absence of a distinct though delicate dried funiculus may be of assistance. In snapped cotton examination of the bur to ascertain the number of points of ovular attachment for a particular lock may help decide questionable cases. Positive identification is not always possible, particularly in ginned lint and products of manufacturing processes.

Cotton samples representing different varieties, locations and seasons of growth, and numbers of ovules per lock and of locks per boll were examined to ascertain some of the factors affecting the formation of false motes. The abundance of these structures in a sample was reported as the percentage of locks in which these bodies occurred.

The number of false motes formed was found to vary strikingly among the varieties studied and was shown to be influenced to a marked degree by environmental factors, especially those associated with seasonal differences. False motes were much more abundant in five- than in four-lock bolls, and within varieties the percentage of locks in which they were found tended to decrease with an increase in the number of ovules per carpel.

The failure to recognize false motes as distinct from true motes, or aborted seeds, may constitute a source of error in estimating for a particular sample of seed cotton the percentage of the ovules that failed to mature (formed motes).

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BACTERIAL LEAF BLIGHT AND STALK ROT OF CORN[1]

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INTRODUCTION

A bacterial disease of dent corn (*Zea mays* L.) different from any previously described has occurred sporadically in several Southern and Central States in the past 20 years. It was reported first from Alabama (*5*).[2] In 1928, specimens of the diseased corn were received from S. H. Gibbons, county agent of Baldwin County, Ala., and the disease was found in Virginia shortly thereafter in the same year. In later years it was found in Georgia, Texas, Kansas, and Nebraska (*6*, *11*, *19*, *20*, *21*). Fortunately, the disease has never assumed major importance, although in a few cases it has been rather severe in localized areas. A very similar disease has been reported from Australia by Ludbrook (*10*).

Most varieties of dent corn seem to be rather resistant, whereas some lines of popcorn (*Zea mays* var. *everta* (Sturtev.) Bailey) have been reported as especially susceptible.[3] Sweet corn (*Z. mays* var. *saccharata* (Sturtev.) Bailey) also is susceptible. The purpose of the present paper is to describe the disease more fully and to report the identification of the causal organism.

DESCRIPTION OF THE DISEASE

The disease occurs usually in localized areas in the field, as a leaf blight and as a rot of the upper part of the stalks. Both manifestations may occur on the same plants.

On the leaves the lesions are variously scattered and rather sharply delimited; they range from small, elliptical or oblong spots about 1 by 2 millimeters to long, necrotic stripes 2 to 5 by 100 to 400 millimeters or longer (pl. 1). These longer lesions may coalesce and form extensive necrotic areas that may involve the entire width of leaves or considerable portions of them. Later the badly diseased leaves may become much shredded (fig. 1, *A* and *B*), especially in stormy weather.

[1] Received for publication December 29, 1948.

[2] Italic numbers in parentheses refer to Literature Cited, p. 731.

[3] S. M. Pady in correspondence.

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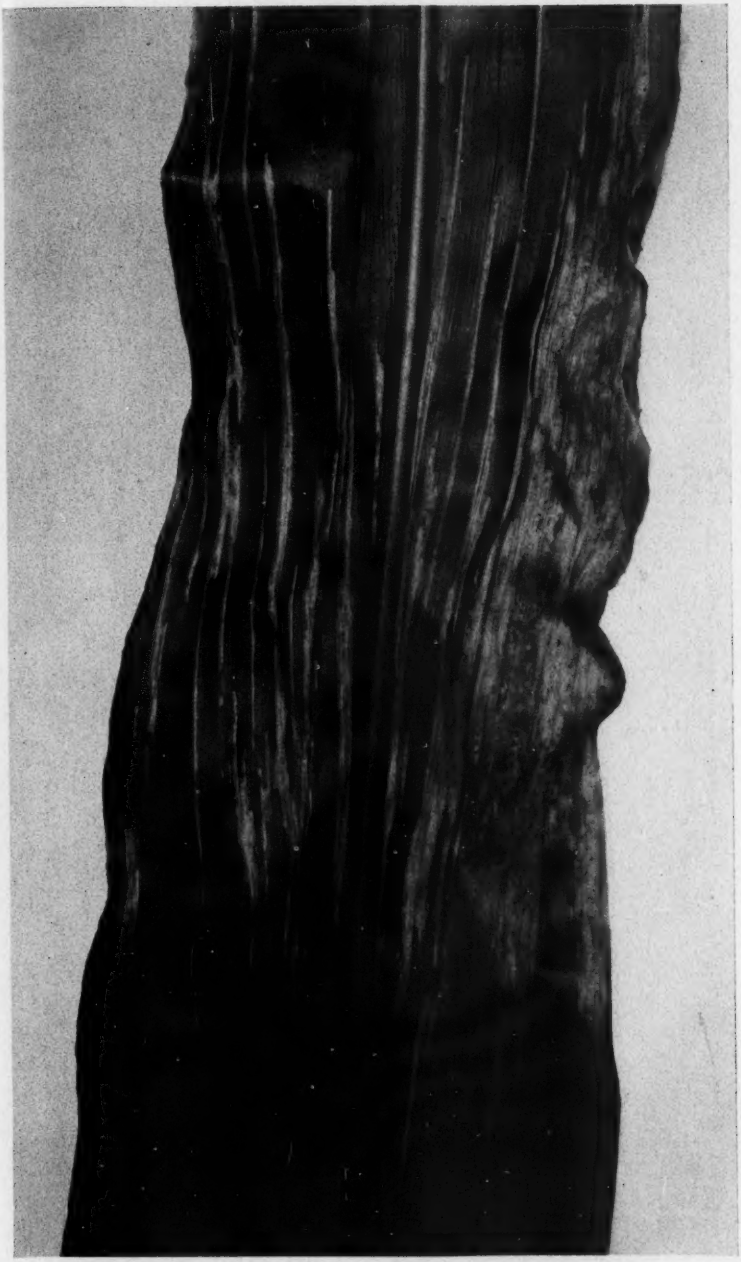
FIGURE 1.—Shredding of corn leaves naturally infected with the leaf blight and stalk rot bacterium: *A*, Early stage, after a heavy rain, Arlington Experiment Farm, Arlington, Va.; *B*, extreme stage, Bay Minette, Ala.

At first the lesions are water-soaked and "olive-green";[4] later the tissues of their centers collapse and bleach first to "warm buff" and later to "light buff" with a delicate edging of "sepia" to "cinnamon-buff." All the leaf lesions are more or less translucent. Sections from leaf lesions examined microscopically in water show abundant streaming of motile bacteria.

The stalk rot occurs usually at or just above the point where the ears are produced, causing a dark-brown to black rot of the internodes and nodes (fig. 2). The outside of the stalk may be discolored with

[4] Throughout the paper, quoted colors are from Ridgway (*12*).

Bacterial Leaf Blight and Stalk Rot of Corn



Upper surface of corn leaf naturally infected with the leaf blight and stalk rot bacterium, showing characteristic lesions, Arlington Experiment Farm, Arlington, Va.

FIGURE 2.—Interior of the upper portion of a cornstalk naturally infected with the leaf blight and stalk rot bacterium, showing the rotting above the ear node, Bay Minette, Ala.

brownish-red streaks. Inside, the rot is black and usually without a particularly foul odor. The pith tissue becomes shredded, as the rot destroys the parenchyma, and leaves the bundles more or less intact. As the rot progresses, the tops die and bleach just before or at the time the tassels emerge (fig. 3, *B*). Most of the plants thus attacked are dwarfed and often develop multiple ears (fig. 3, *A*), all of which are usually sterile and some of which may become rotted.

FIGURE 3.—Tops of corn plants naturally infected with the leaf blight and stalk rot bacterium, showing leaf lesions and multiple ears (*A*) and leaf lesions and killed, bleached top (*B*); both from Arlington Experiment Farm, Arlington, Va.

THE ORGANISM

ISOLATION

The organism has been isolated repeatedly from leaf lesions and also from rotted stalks. Lesions on the leaves were surface-sterilized in 1:1,000 mercuric chloride solution for 30 seconds, washed in sterile water, shredded on a sterile slide, and put into beef-peptone broth. After a few minutes, dilution plates from this were made, with beef-peptone agar as the medium. Isolations from rotted stalks were made by aseptically cutting out bits of the infected tissue along the margins and shredding them into beef-peptone broth. Dilution plates from this were made, with beef-peptone agar as the medium. All plates were stored at room temperature (about 25° to 30° C.), and within 48 hours they showed an abundance of small white bacterial colonies that appeared almost in pure culture. Transfers were made from single colonies.

CULTURAL CHARACTERS

Unless otherwise stated, the incubation temperature at which the organism was grown was 30° C. and the reaction of the media was about pH 7.

Beef-peptone agar plates.—In dilution plates, after 48 hours colonies of the organism freshly isolated from the host are circular, 1 to 4 millimeters in diameter, convex, smooth, white by reflected light, bluish white by obliquely transmitted light, glistening, with margins entire or becoming irregular. In most cases there is a fine cross hatching throughout the colony, but in others there are not definite internal markings. Most of the colonies show a definite clearing of the medium immediately around them; this is accentuated by a heavy clouding of the medium at the outer edge of the cleared area. Others show no clearing. As the medium dries out the colonies become almost invisible.

The smooth colonies that were isolated first were not stable and eventually dissociated into smooth and rough colonies of several types. Some of the rough colonies were irregular in outline, glistening, with or without clearing of the medium, and without characteristic internal markings. Others were round, slightly pebbly rough on the surface, and glistening with coarsely cross-hatched internal markings and slightly fimbriated margins extending into a cleared area around them. Some colonies had a pronounced thickening in the center, which caused a noticeable hump on the surface, coarse inner markings, and a clearing of the medium around them. The margins of many of the smooth colonies were entire at first and later became irregularly lobed or definitely fimbriated, extending in a lacelike pattern into the cleared portion of the medium.

Beef-peptone agar slants.—Growth after 48 hours is moderate, filiform, glistening, white to bluish white, with margins entire or becoming undulated and thin, and in most cases with noticeable clearing of the medium surrounding the streak. The surface ranges from smooth to pebbly rough.

Beef-peptone broth.—After 24 hours cultures show heavy clouding and moderate sediment, which disperses throughout the liquid in slightly stringy whorls when the tube is gently agitated. In older cultures the sediment is heavier and more stringy and clings to the bottom of the tube. There is no pellicle or ring.

Potato cylinders.—After 48 hours there is a wet almost invisible growth and the medium is firm but buff-colored above the water. After 10 days the growth is heavier, dark cream to light brown in color, creamy in texture, and rugose, and the part of the potato cylinder above the water is light brown and softened.

Uschinsky's solution (*9*).—Growth is very heavy after 48 hours, with a heavy pellicle, which falls easily to the bottom of the tube when it is agitated and leaves a slight, irregular ring on the tube at the top of the liquid. At the end of 3 weeks the surface growth is so heavy that strands hang down from the pellicle almost to the bottom of the tube. There is no change in the color of the medium.

Fermi's and Cohn's solutions (*9*).—There is no growth at 20°, 25°, 30°, or 35° C.

PHYSIOLOGICAL CHARACTERS

Carbon metabolism.—The slightly modified formula for Ayers, Rupp, and Johnson's synthetic carbohydrate media (*23*) was used. The alcohols and sugars were added at a concentration of 1 percent and the media were sterilized by steaming 1 hour in an Arnold sterilizer on each of three successive days. Before sterilization bromcresol purple was added as an indicator. The color change from purple to yellow was used as the index of fermentation. An accurate final change in the pH was recorded by the use of a Beckman pH meter. Durham fermentation tubes were used so that the production of gas could be observed at the same time. Six representative cultures, inoculated in duplicate, were used in the tests, which were made three times. Acid was produced with all of the carbohydrates tested, namely, glycerol, saccharose, insulin, raffinose, lactose, maltose, dextrose, galactose, mannitol, and levulose. Gas was not produced.

Temperature relations.—The optimum temperature for growth is 30° C. The maximum temperature at which tests were made with beef-agar slants was 40°. The bacterium grew at this temperature. There was slight growth at 10°, but none at 5°. The thermal death point is 52°.

Relation to free oxygen.—Growth occurs only at the surface or just under the surface of rolled cultures in beef-agar tubes, indicating that the bacterium is an aerobe.

Nitrate reduction.—When Bacto-beef-peptone agar with 0.1-percent potassium nitrate added is the medium for growing the bacterium, there is a rapid reduction of nitrate.

Indole production.—Indole is not produced when the organism is grown in Bacto-tryptone broth. The Ehrlich-Böhme and Gnezda methods (*24*) both gave negative tests.

Ammonia production.—Ammonia is produced in beef-peptone agar and beef-peptone broth. Tests with Nessler's solution were positive after 2 days' growth.

Hydrogen sulfide.—When the test-strip method of ZoBell and Feltham and also the lead acetate agar test (*24*) were used, hydrogen sulfide was formed to a very moderate degree.

Hydrolysis of starch.—Starch is hydrolyzed. Streak inoculations on beef-peptone agar containing 0.2 percent soluble starch grew slowly, but after 10 days a clear zone showed outside the area of growth when the plates were flooded with Lugol's iodine.

Reaction in milk.—Milk is slowly peptonized without coagulation. After 7 days there was a complete reduction of litmus in litmus milk, and after 1½ months the deep-blue color of the litmus had returned. When bromcresol purple was used as an indicator, there was a gradual increase of the purple color, indicating increase in alkalinity. After a month the milk had become completely peptonized, leaving a stringy sediment in the bottom of the tube. No curd was formed.

Gelatin liquefaction.—Gelatin is liquified. On Bacto-nutrient gelatin plates at 25° C., there was definite liquefaction by all cultures after 5 days. Utilization of gelatin was demonstrated also by a modified method of Frazier (*4*) in which 4-percent gelatin was used in beef-peptone agar plates. In test tubes liquefaction was too slow to be detected.

MORPHOLOGY

The bacteria are in the form of short rods, rounded at ends, 0.3μ to 0.7μ by 0.6μ to 1.5μ, averaging 0.6μ by 1.0μ. These ranges and averages are based on the measurement of 50 cells from each of 6 different cultures stained with Ziehl's carbolfuchsin. The rods occur singly, in pairs, or in chains of 3 to 10 cells; they are motile by a single polar flagellum, demonstrated by Casares-Gil's staining method. Capsules can be seen by the use of either Anthony's method or Huntoon's method (*22*). There are no spores, and the cells are not acidfast. The bacteria are Gram-negative when the cultures are grown on beef agar for 1 to 4 days.

INOCULATIONS

The pathogenicity of the bacterium isolated repeatedly from diseased leaves and stalks of corn from different areas was established by artificial inoculations on the following hosts and reisolations of the typical organism from them: Illinois Yellow Dent and Alabama Strawberry dent corn, Golden Bantam and Adams Extra Early sweet corn at Washington, D. C., and Arlington Experiment Farm, Arlington, Va., and U. S. Hybrid 13 dent corn, Golden Giant and Golden Cross Bantam sweet corn, Fulcaster wheat (*Triticum aestivum* L.), Appler oats (*Avena sativa* L.), Oderbrucker barley (*Hordeum vulgare* L.), Abruzzes rye (*Secale cereale* L.), Honey sorgo (*Sorghum vulgare* Pers.), Tift Sudan grass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.), *Setaria lutescens* (Weigel) F. T. Hubb. (*Chaetochloa lutescens* (Weigel) Stuntz), and *S. geniculata* (Lam.) Beauv. (*C. geniculata* (Lam.) Millsp. and Chase), at Plant Industry Station, Beltsville, Md. No experiments were conducted to determine the relation of temperature to infection, but the best infections were obtained when greenhouse temperatures were relatively high, about 85° to 95° F. When the temperature was as low as 70° there was little or no infection, and infection was only slight when the temperature was about 75°.

For the inoculations in the greenhouse the plants were grown to the 4- or 5-leaf stage in 4-inch pots usually with 5 corn plants or about 8 to 10 or more small-grain or grass plants per pot. In each inoculation on each host with each culture, the plants in 1 pot were inoculated and corresponding plants in at least 1 pot were similarly treated with sterile water and similarly incubated as controls. In the inoculations in the field, at least 5 corn plants of each variety or strain in the pretasseling stage were inoculated with each culture thus used and at least 5 corresponding plants were similarly treated with sterile water as controls.

Infections were readily obtained by spraying the leaves with water suspensions of the bacterium and holding the plants in a moist chamber for 24 hours. Symptoms appeared within 48 hours when inoculations of this type were made in the greenhouse (fig. 4, *A* and *B*; fig. 5). Corn seedlings were inoculated also by injecting the inoculum into their centers through the leaf sheaths. A summary of the pathogenicity studies of leaf blight and stalk rot at Beltsville is given in table 1.

The lesions produced on corn were typical of those that occur under natural conditions. Young lesions on the small grains appeared as green water-soaked areas surrounded by yellowish tissue. Later they became dark olive-green surrounded by yellowed tissue sometimes bordered with "sepia." Older lesions became buff or light brown, surrounded with yellow and red. In severe infections the leaves sometimes fell over about one-third to one-half the distance from the tip.

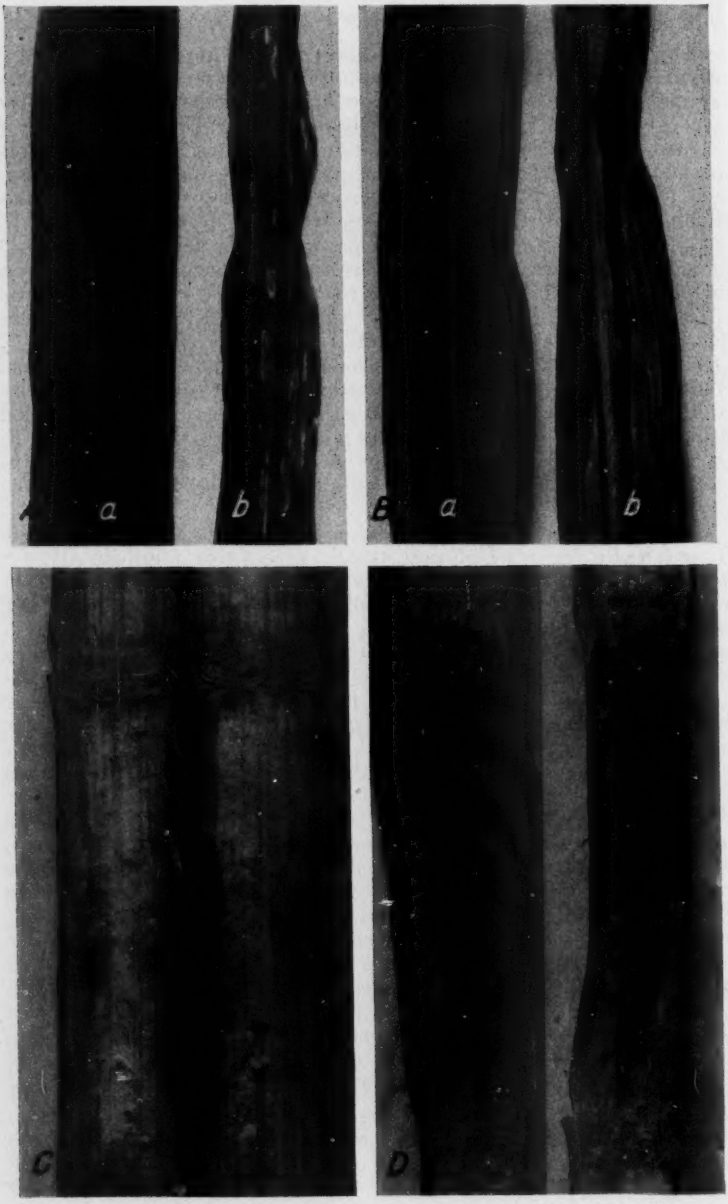


FIGURE 4.—Leaves of dent corn (*A*) and sweet corn (*B*) grown in the greenhouse: *a*, Uninoculated control; *b*, inoculated with the leaf blight and stalk rot bacterium. Split stalks of dent corn grown in the field: *C*, Control, injected hypodermically with sterile water; *D*, inoculated hypodermically with the leaf blight and stalk rot bacterium.

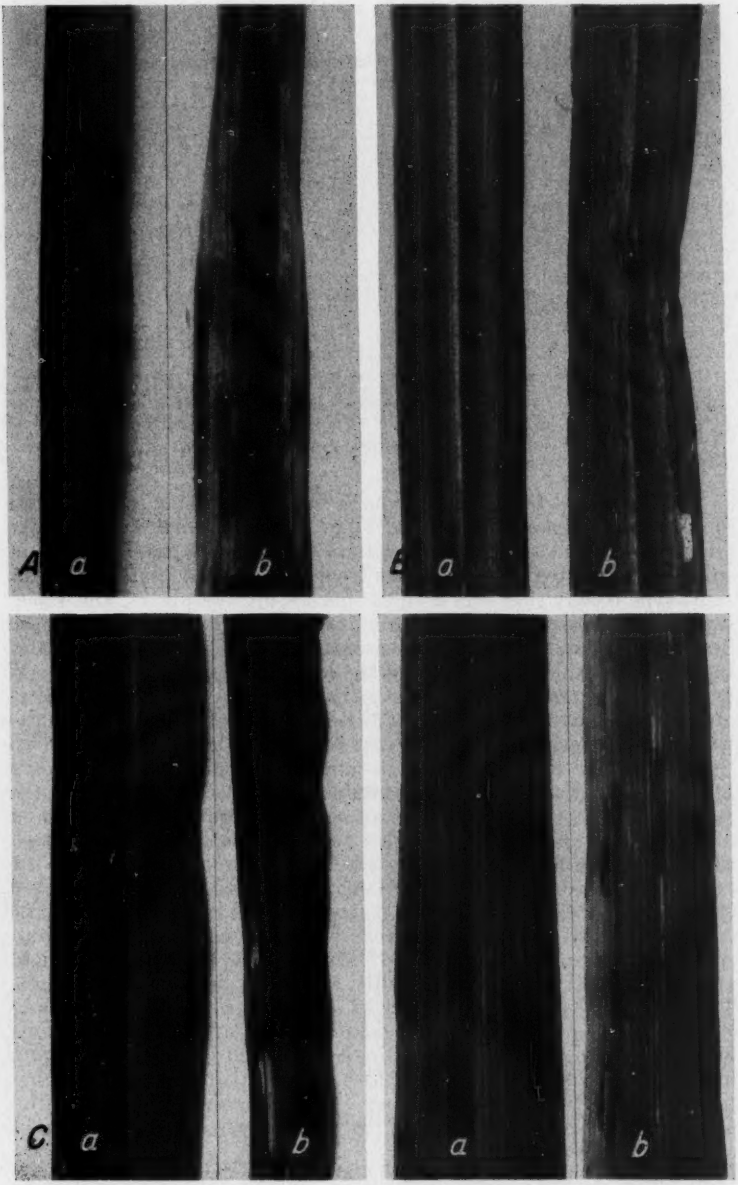


FIGURE 5.—Leaves of wheat (*A*), barley (*B*), Sudan grass (*C*), and *Setaria lutescens* (*D*): *a*, Uninoculated control; *b*, inoculated in the greenhouse with the leaf blight and stalk rot bacterium, showing characteristic lesions.

TABLE 1.—*Summary of infections in the greenhouse on various hosts by 9 cultures of the organism isolated from lesions on corn leaves, Beltsville, Md.*

[Infection ratings: 0, None; 1, trace; 2, slight; 3, moderate; 4, heavy; and 5, very heavy]

Plant inoculated	Infection rating from indicated culture [1]								
	2a	2aD1	2aD3	2aD3 [2]	2aD3r [2]	2b	$2bS_1$	3a	3ar [2]
U. S. Hybrid 13 dent corn	3	5	4	4	3	2	5	4	4
Golden Cross Bantam sweet corn			4	4	4		5	4	5
Golden Giant sweet corn	5	5	5			5	5	5	
Fulcaster wheat	4	4	5	2	2	5	5	5	2
Appler oats	4	4	5	2	3	5	5	5	1
Oderbrucker barley	4	4	5	4	4	5	5	5	4
Abruzzes rye	4	4	5	3	3	5	5	5	5
Honey sorgo	1	1	2	4	4	2	2	2	3
Tift Sudan grass	1	2	2	3	5	1	1	2	3
Setaria lutescens	2	3	2	4	4	1	1	1	4
Setaria geniculata	1	1	0	3	3	1	1	0	3

[1] Cultures 2a, 2b, and 3a were isolated from different lesions on leaves of U. S. Hybrid 13 dent corn received from Kansas, July 1943; cultures 2aD1 and 2aD3 from infections resulting from artificial inoculations with culture 2a on U. S. Hybrid 13; culture $2bS_1$ from infection resulting from artificial inoculation with culture 2b on Golden Giant sweet corn; and cultures 2aD3r and 3ar, reisolations from infections on Golden Cross Bantam by 2aD3 and 3a, respectively.

[2] Used in a separate series of inoculations in which infection ratings on some hosts were somewhat different rom those previously obtained.

When cultures from the previously described variant colonies were sprayed on leaves of seedlings of U. S. Hybrid 13 dent corn, Golden Giant sweet corn, and *Setaria lutescens* as described previously, typical lesions resulted. Reisolations from the infected plants gave the same-type colony that was used in inoculating the plants, and sometimes one or two of the other types of variant colonies that have been described, as well as the typical, smooth colonies. This apparently indicates that the organism is not stable and is represented by several types that are pathogenic and may change within the host as well as on artificial media. Throughout these various series of leaf inoculations, the uninoculated control plants remained healthy.

Stalk inoculations were made in the field and in the greenhouse by hypodermic injections of water suspensions of the bacterium into the stalks of plants just before the ears began to form. Injections were made as near to the upper ear node as could be determined. Fourteen different varieties of open-pollinated dent corn were inoculated in the field at Arlington Experiment Farm, Arlington, Va. These showed typical stalk rot (fig. 4, *C* and *D*), top rot, and multiplicity of ears. All were about equally susceptible. Inoculations were made into stalks of dent corn, U. S. Hybrid 13 and U. S. Hybrid 357, in the field at Plant Industry Station, Beltsville, Md., with six different cultures. Typical stalk rot and multiplicity of ears resulted, but no top rot occurred in any of the plants inoculated. Similar stalk inoculations were made into three dent corn single crosses grown in 14-inch pots in the greenhouse with seven cultures. Typical stalk rot and multiplicity of ears were produced in each of the crosses even though the plants did not reach maturity in the greenhouse. The uninoculated controls remained healthy.

One limited series of inoculations also was made on seed. Fifteen kernels each of U. S. Hybrid 13 dent corn and Golden Giant sweet corn were inoculated by soaking them for 15 minutes in a water suspension of each of seven cultures of the leaf blight and stalk rot or-

ganism. These inoculated kernels were planted in a warm greenhouse in 6-inch pots of autoclaved soil, five kernels per pot. In 6 days the seedlings were in the three-leaf stage and some of them showed water-soaked lesions in the leaves. Later, a number of other plants of both the dent and the sweet corn showed conspicuous lesions. The final results, taken 12 days after planting, are given in table 2. Reisolations were made from representative lesions in the leaves of the U. S. Hybrid 13 and Golden Giant plants inoculated with each culture, and the characteristic organism was recovered in each case. All of the uninoculated, control plants remained healthy.

TABLE 2.—*Results from inoculating seed of U. S. Hybrid 13 dent corn and Golden Giant sweet corn with water suspensions of 7 cultures of the leaf blight and stalk rot organism*

Culture	U. S. Hybrid 13			Golden Giant		
	Planted	Emerged	Infected	Planted	Emerged	Infected
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
2a	15	15	27	15	12	42
2b	15	15	27	15	13	77
2c	15	15	60	15	15	60
2d	15	14	21	15	13	46
2e	15	15	47	15	14	50
2f	15	14	36	15	15	53
3a	15	15	40	15	12	58
None (control)	15	15	0	15	15	0

Two series of inoculations were made on vegetables. In each of these, six cultures of the organism previously found to be pathogenic on corn and other hosts were tested for pathogenicity on fresh, clean slices of cabbage, carrot, potato, and turnip. At least four slices of each of these were cut carefully with a sterile knife and placed in each of two large petri dishes. One set was left uninoculated and the other was inoculated by placing a drop of water suspension of each culture separately on the freshly cut surface of each vegetable. Within 5 days a distinct soft rot, with a foul odor, was produced on each of the vegetables. The cultures showed only minor differences in their ability to cause rot. The uninoculated control slices remained sound. Isolations were made from each of these inoculation tests and the typical organism was obtained in each case in which infection occurred.

IDENTIFICATION

The organism described as causing the bacterial leaf blight and stalk rot of corn has been found to agree in all essential respects with that described by Rosen (*14*, *16*) as *Pseudomonas alboprecipitans*. While Rosen described this species as the cause of a bacterial disease of *Chaetochloa lutescens* (*Setaria lutescens*) as found in nature, he reported that under greenhouse conditions it was pathogenic also on *C. geniculata* (*S. geniculata*), *Holcus sorghum* L. (*Sorghum vulgare*), *H. sorghum sudanensis* Hitchc. (*S. vulgare* var. *sudanense*), *Hordeum vulgare*, *Secale cereale*, *Triticum vulgare* Vill. (*T. aestivum*), and *Zea mays*.

As stated previously, the bacterial leaf blight and stalk rot on dent corn occurs in nature in a number of Southern and Central States.

The organism isolated was shown to be pathogenic on essentially the same hosts reported as susceptible to *Pseudomonas alboprecipitans* by Rosen.

In addition to the similarity in host range, the cultural, physiological, and morphological characters of the organism under study are in essential agreement with those of *Pseudomonas alboprecipitans* Rosen (*16*) (*Bacterium alboprecipitans* Rosen (*16*), *Phytomonas alboprecipitans* (Rosen) Bergey et al. (*2*)).

COMPARISON WITH OTHER BACTERIAL DISEASES OF CORN

The bacterial leaf blight and stalk rot of corn caused by *Pseudomonas alboprecipitans* as described differs distinctly from baterial wilt caused by *Bacterium stewarti* E. F. Sm. in both symptoms and causal organism. In the former the lesions on the leaves are definite spots or stripes, usually with a "sepia" margin; the stalk rot is limited to the upper part of the plant; and the causal organism is white. In the latter the leaf lesions tend to be long and rather narrow with wavy margins, streaking the leaves. From these lesions the bacterium spreads to the stalk, especially in sweet corn, produces a systemic infection, chiefly in the vascular system, and is yellow.

The conspicuous difference between the stalk rot of corn caused by *Pseudomonas alboprecipitans* and that described by Rosen (*13*, *15*, *17*, *18*) as caused by *Phytomonas dissolvens* Rosen is that the former is confined to the upper part of the stalks and the latter to the lower part. While both of these organisms are white, they differ in certain physiological characters, particularly as regards gas formation. *P. dissolvens* grows more rapidly, forms large colonies, and produces gas in various carbohydrate media, whereas *Ps. alboprecipitans* forms smaller colonies and no gas.

The bacterial stalk rot described by Ark (*1*) as caused by *Phytomonas lapsa* Ark (*Pseudomonas lapsa* (Ark) Burk. (*3*)) differs distinctly from that caused by *Ps. alboprecipitans* in that the corn plants attacked by the former commonly fall to the ground and the causal bacterium produces fluorescence in Uschinsky's, Fermi's, and Cohn's solutions.

The bacterial disease on corn described by Kendrick (*7*, *8*) as caused by *Bacterium holci* Kendrick (*Pseudomonas holci* Kendrick) occurs only on leaves as small circular, elliptical, or irregular spots, at first water-soaked and later brown with a narrow darker brown to reddish-brown border. The organism, which is now considered the same as *Ps. syringae* van Hall (*3*), is white and fluorescent and produces a greenish pigment in some media; therefore it is distinctly different from *Ps. alboprecipitans*.

Nonparasitic injuries to corn leaves caused by sodium nitrate having been accidentally thrown into the leaf whorl on hasty examination may sometimes be confused with leaf lesions caused by *Pseudomonas alboprecipitans*. While the sodium nitrate may cause bleached spots and stripes, these usually do not show the characteristic "sepia" borders; nor do they show bacterial streaming when examined microscopically. Likewise, if plated, they do not yield the characteristic bacterium.

DISCUSSION

On account of the sporadic occurrence of leaf blight and stalk rot and the lack of specific information on the source of inoculum in

nature, no study of control measures was made. From the standpoint of economic importance thus far, control measures seem scarcely needed. It is not known, however, how severe the disease might become under some set of conditions especially favorable for its development. Therefore the disease should be watched.

While it has been shown that the pathogen may be seed-borne if inoculated onto the seed just before planting, there is no information to indicate that it is seed-borne under natural conditions. On the other hand, the pathogen has been identified as *Pseudomonas alboprecipitans*, which was originally isolated from *Setaria lutescens*, a common weed in cornfields. There is the possibility, therefore, that the pathogen may spread from this host to corn under field conditions. Since the identification of the pathogen there has been no opportunity to make field observations on this point.

SUMMARY

A bacterial leaf blight and stalk rot of corn has been found to occur sporadically in various Southern and Central States, where it has been of minor importance.

The disease attacks both the leaves and the stalks of corn.

The causal organism is a white, Gram-negative, motile bacterium identified as *Pseudomonas alboprecipitans* Rosen.

Inoculation experiments have shown that the disease may be readily induced on corn, wheat, rye, barley, oats, and *Setaria lutescens* by spraying the leaves with water suspensions of the bacterium. Light infections were obtained in the same way on *S. geniculata*, Honey sorgo, and Sudan grass.

Stalk infection occurred when water suspensions of the organism were inoculated into the stalks of corn previous to tasseling.

Kernels of both dent corn and sweet corn soaked in water suspensions of the bacterium and then planted in sterile soil in the greenhouse produced infected seedlings, while corresponding controls remained healthy.

Variants of the typical colonies showed pathogenicity on corn and *Setaria lutescens*.

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SYMPTOMS OF AMINO ACID ACTION ON TOBACCO SEEDLINGS IN ASEPTIC CULTURE[1]

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INTRODUCTION

Data reported in an earlier paper[2] indicated that traces of *dl*-isoleucine in the cultural medium caused specific changes in gross morphology of tobacco seedlings closely approximating those of the disease known as frenching. The abnormalities observed included strap leaves, reticular chlorosis, inhibition of stem elongation, and large increase in leaf number. Growth responses to other amino acids were likewise specific. Wide variations in gross morphology were found to take place with changes in concentration and with admixture of different amino acids. The suggestion was therefore made that the symptoms of frenching are primarily localized responses to toxic concentrations of free amino acids in the tissues of the plant. In this case break-down of protein metabolism in the plant because of malnutrition could lead to a variety of growth responses depending on the kind and concentration of intermediate metabolic products and the regions in which they accumulated.

Research along these lines is necessarily limited because of unavailability of peptides in pure form. It is probable, also, that not all the natural amino acids and similar compounds of the tobacco plant are as yet known. In a continuation of such work, however, seedlings have been grown to greater size by use of larger flasks, and a distinction has been made between the actions of the natural and unnatural optical isomers of isoleucine. A few organic compounds not previously reported on have also been tested. An effort, lastly, has been made to distinguish between normal and abnormal hormone effects of these plant metabolites. Any response within the range of normalcy of the plant would indicate that gross morphology is, in part at least, a reflection of protein metabolism.

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[1] Received for publication December 16, 1948.

[2] STEINBERG, R. A. GROWTH RESPONSES TO ORGANIC COMPOUNDS BY TOBACCO SEEDLINGS IN ASEPTIC CULTURE. Jour. Agr. Res. 75: 81–92, illus. 1947.

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EXPERIMENTAL TECHNIQUE

Seedlings of Maryland Medium Broadleaf tobacco (*Nicotiana tabacum* L.)—Robinson strain—were grown with continuous illumination from 3,500° C. white fluorescent lamps. The plants were propagated aseptically in the manner previously described.[3] Some were grown in 200-milliliter Erlenmeyer flasks with 50 milliliters of a complete mineral agar, at 25° C. and an illumination intensity of about 500 foot-candles. Others were grown in 1-liter Erlenmeyer flasks with 250 milliliters of a complete mineral agar, at room temperature. Illumination at the agar surface in the larger flasks was about 300 foot-candles, because of greater distance from the lamps.

Tests with the larger flasks were made individually, in order to conserve materials; those with the small flasks were made in duplicate. In general, trials were repeated with decreasing concentrations of a compound until a value was found that caused a moderate alteration of gross morphology.

RESPONSES TO AMINO ACIDS

Table 1 presents the results for seedlings grown in liter flasks for 2 months with nutrient agar to which various amino acids had been added in various concentrations and for a seedling grown in a 2-liter flask for 4 months with nutrient agar to which 200 p. p. m. (parts per million) of *dl*-isoleucine had been added. The concentrations listed indicate the degrees of effectiveness of the individual compounds in bringing about responses. A few compounds, such as *dl*-alanine and *l*-hydroxyproline, were effective in high dilution. Others, as *d*-arginine, *l*-cystine, and *dl*-ornithine, caused no reaction at relatively high concentration. The symptoms of excessive amino acid concentration differed widely according to which acid was used. The effects of admixture of a second amino acid—*l*-hydroxyproline, *l*-leucine, or *l*-tryptophane—on the growth response to *dl*-isoleucine varied with the kind and quantity of admixed acid.

Symptoms of amino acid action listed in table 1 include most of those observed in short-duration experiments already reported by the writer. The longer growth periods, however, permitted a greater degree of expression, because of larger size of plant parts. Some of the symptoms fell within the range of normality; that is, they could have been mistaken for seasonal or varietal differences. Use of *dl*-norleucine led to new responses under a 2-month duration—thick leaves with irregular or ragged margins, and down-cupped leaves with hooked tips.

Figures 1–8, photographs of some of the plants described in table 1 and of other plants used in the experiments, illustrate the wide differences in morphology resulting from the presence of excessive concentrations of different amino acids alone or in mixture. Figure 1 reveals the closeness of approximation to the symptoms of frenching brought about with *dl*-isoleucine. Inhibition of stem growth, repression of leaf lamina development, reticular mottling, and large increase in leaf number are all clearly evident. The down-cupped leaves of the plant in figure 2, which was grown in a medium contain-

[3] See footnote 2.

TABLE 1.—*Effects of amino acids on gross morphology of tobacco seedlings—Maryland Medium Broadleaf (Robinson strain)—grown for 2 months in 1-liter Erlenmeyer flasks under aseptic conditions*

Treatment	Quantity	Appearance of seedling
	Parts per million	
Tests of single compounds:		
No treatment		Normal.
Adenine	50	Transient, down-cupped leaves, dark mottle.
dl-Alanine	5	Light rims, marginal spots, basal leaves with yellow spices.
dl-α-Aminobutyric acid	10	Tiny white seedlings. (Later they became normal.)
d-Arginine hydrochloride	400	Light interveinal chlorosis, surface roots.
l-Asparagine	36	Marginal chlorotic leaf spots.
l-Aspartic acid	50	Marginal leaf spots and slight cupping.
l-Cystine	400	Normal.
d-Glutamic acid	30	Chlorotic leaf blotches.
Glycine	45	Chlorotic spots and blotches.
l-Histidine dihydrochloride	50	Pale leaf rims.
l-Hydroxyproline	3	Cupped leaves, normal stem growth.
dl-Isoleucine [1]	200	Leaf mottle and strapping; rosette; growth of axillary leaves only.
l-Leucine	300	Dark primary leaf veins; yellow basal leaves.
d-Lysine dihydrochloride	50	Normal.
dl-Methionine	200	Dark-green rosette; puffy, down-cupped leaves; yellow basal leaves.
dl-Norleucine	200	Dark-green rosette; thick leaves; leaf margins irregular; down-cupped, hooked tips; yellow basal leaves.
dl-Ornithine hydrochloride	400	Normal.
dl-β-Phenylalanine	300	Severe mottle, dead basal leaves, large necrotic blotches.
l-Proline	45	Transient rim mottle; yellow basal leaves.
dl-Serine	40	Transient leaf mottle.
dl-Threonine	45	Transient stem retardation; short leaf midribs.
l-Tryptophane	20	Narrow, pointed leaves.
l-Tyrosine	300	Dark-green leaves with reticular mottle; black roots.
dl-Valine	20	Yellow basal leaves.
Tests of mixtures:		
dl-Isoleucine in mixture	200	
l-Leucine admixture	40	Few leaves, some narrow; reticular chlorosis.
l-Hydroxyproline admixture	5	Strapped, reticulated leaves.
l-Tryptophane admixture	4	Many reticulated strap leaves; rosette.
	8	Many reticulated leaves; rosette; suckers.
dl-Isoleucine alone	200	Transient strap leaves; many narrow, reticulated leaves; rosette.
Tests of subtoxic concentrations of *dl*-isoleucine.	0	Normal; height on 39th day 5.2 inches.
	2	Normal; height on 39th day 4.5 inches.
	4	Normal; height on 39th day 4.0 inches.
	5	Normal; height on 39th day 2.0 inches.
	20	Slightly reticulated leaves; height on 39th day 2.0 inches.

[1] Growth period 4 months, flask capacity 2 liters.

ing 3 parts per million of *l*-hydroxyproline, resemble those shown by Valleau and Johnson in their paper on frenching.[4] This amino acid is one of those most effective in their action on the tobacco plant; it is lethal at 5 parts per million. Leaves with irregular margins (fig. 3) have been noticed only in the presence of *dl*-norleucine. Arching backward of the young bud leaves is characteristic of the action of this same compound. The effect of *dl*-methionine (fig. 4) seems to be largely an inhibition of midrib and veinal growth, resulting in leaf twisting and puffiness. Otherwise the plant seemed vigorous and healthy.

Some of the effects of the presence of other amino acids on the action of *dl*-isoleucine on tobacco seedlings appear in figures 5–8. Figure 5 shows the effect on growth of a mixture of 5 parts per million each of *dl*-isoleucine and *l*-hydroxyproline. The narrow, badly hooked

[4] VALLEAU, W. D., AND JOHNSON, E. M. TOBACCO FRENCHING—A NITROGEN DEFICIENCY DISEASE. Ky. Agr. Expt. Sta. Bul. 281, pp. 175–253, illus. 1927.

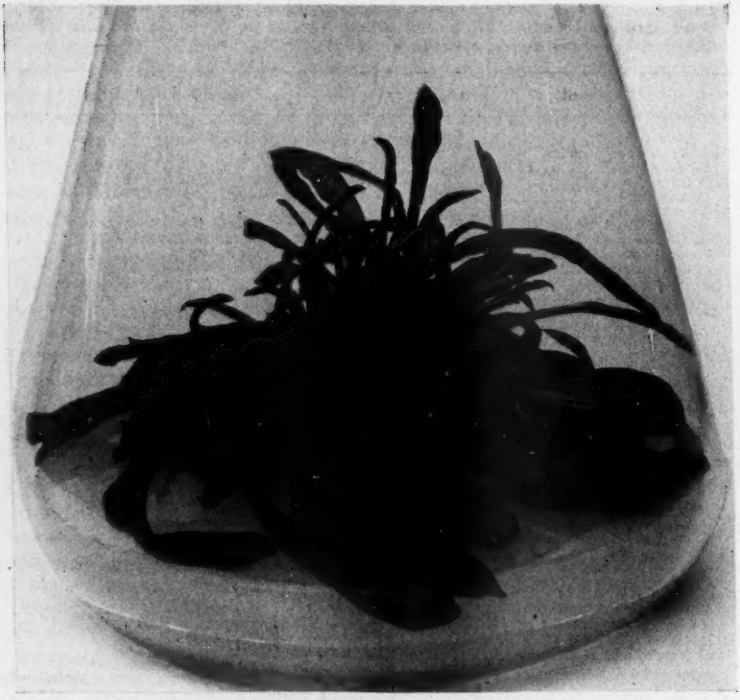

FIGURE 1.—Seedling of Maryland Medium Broadleaf tobacco grown for 4 months in a 2-liter flask with a mineral-salt agar containing 200 parts per million of *dl*-isoleucine. The lower leaves became mottled, and the terminal bud ceased to grow. The axillary buds then developed into strap leaves, although branch elongation was practically suppressed.

leaves bear hardly any resemblance to those obtained with separate use of these amino acids (figs. 1 and 2). An example of striking contrast in response to different mixtures of even the same two amino acids is supplied by the seedlings shown in figures 6 and 7, which were grown with 200 parts per million of *dl*-isoleucine and with 4 parts per million and 20 parts per million, respectively, of *l*-tryptophane. The first (fig. 6) was a large and vigorous plant having many strap leaves and having each of its lower leaves tightly rolled so that the lower leaf surface was covered. The second (fig. 7) was a tiny rosette with leaves of almost normal width. Both seedlings exhibited chlorosis.

Use of the mixture 200 parts per million of *dl*-isoleucine plus 20 parts per million of *l*-leucine resulted in inhibition of stem growth and in reticular chlorosis of a more pronounced type (fig. 8). However, it permitted development of leaf width far nearer to normal than that associated with use of *dl*-isoleucine alone. (A higher concentration of *l*-leucine would presumably have further diminished the morphological effects of the *dl*-isoleucine.)

Emphasis has been placed so far on the more marked responses in gross morphology by tobacco seedlings to addition of amino acids. That growth responses to lower concentrations of amino acids may occur without any indication of morphological abnormality in the plant was demonstrated with *dl*-isoleucine (table 1). Concentrations of this acid ranging from 2 to 5 parts per million produced seedlings

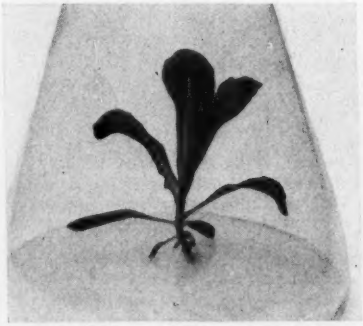

FIGURE 2.— Seedling of Maryland Medium Broadleaf tobacco grown for 2 months in a liter flask with a mineral-salt agar containing sucrose and 3 parts per million of *l*-hydroxyproline. Note characteristic cupping of somewhat narrow leaves.

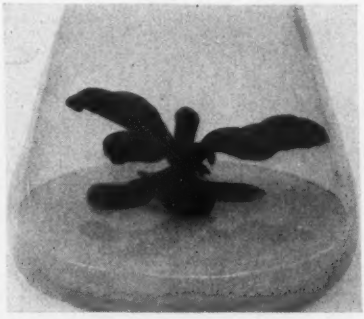

FIGURE 3.—Seedling of Maryland Medium Broadleaf tobacco grown for a month in a liter flask with a mineral-salt agar containing sucrose and 200 parts per million of *dl*-norleucine. A dark-green rosette with slightly cupped leaves having irregular margins and younger leaves having down-curled and hooked tips.

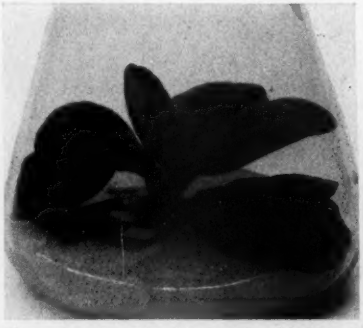

FIGURE 4.— Seedling of Maryland Medium Broadleaf tobacco grown for 2 months in a liter flask with a mineral-salt agar containing sucrose and 200 parts per million of *dl*-methionine. A dark-green rosette with slightly cupped leaves in which inhibition of veinal growth relative to growth of interveinal tissue has produced puffiness.

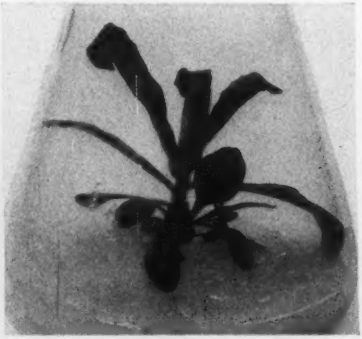

FIGURE 5.— Seedling of Maryland Medium Broadleaf tobacco grown for a month in a liter flask with a mineral-salt agar containing sucrose and 5 parts per million each of *dl*-isoleucine and *l*-hydroxyproline. Note accentuated backward hooking of leaf tips. The leaves are wrinkled and covered with dark blotches.

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FIGURE 6.— Seedling of Maryland Medium Broadleaf tobacco grown for 2 months in a liter flask with a mineral-salt agar containing sucrose, 200 parts per million of *dl*-isoleucine, and 4 parts per million of *l*-tryptophane. The plant is large and vigorous but has many strap leaves and has each of its lower leaves tightly rolled. Reticular chlorosis is present.

FIGURE 7.— Seedling of Maryland Medium Broadleaf tobacco grown for 2 months in a liter flask with a mineral-salt agar containing sucrose, 200 parts per million of *dl*-isoleucine, and 20 parts per million of *l*-tryptophane. Note absence of stem elongation, formation of leaves by axillary buds, and reticular chlorosis of leaves.

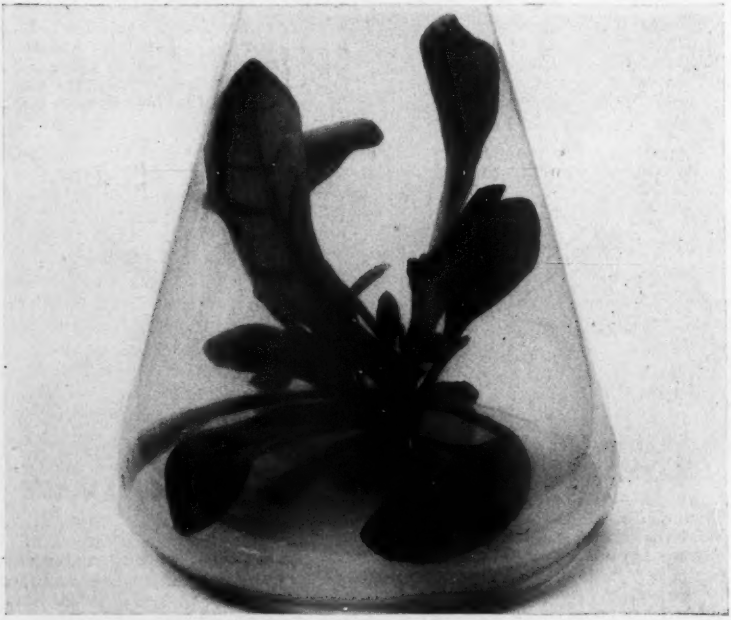

FIGURE 8.—Seedling of Maryland Medium Broadleaf tobacco grown for 2 months in a liter flask with a mineral-salt agar containing sucrose, 200 parts per million of *dl*-isoleucine, and 20 parts per million of *l*-leucine. Note decreased formation of strap leaves and outstanding reticular chlorosis.

that were normal to all appearances except for retardation of growth in height, which varied inversely with concentration of the amino acid.

RESPONSES TO OTHER COMPOUNDS

Table 2 describes seedling responses not only to amino acids but to other compounds that have been reported to be present in the tobacco plant or that may justifiably be assumed to be present in it, observed after only 28 days of growth of the seedlings in 200-milliliter flasks. Acids of high molecular weight were found less effective in bringing about growth responses of tobacco seedlings than amino acids. Acids of low molecular weight, including formic, acetic, and acrylic, were as effective as any of the amino acids. Attention is called to the fact that *d*-glutamine at 200 parts per million permitted normal development of the seedlings, whereas *l*-asparagine and *l*-aspartic acid in much lower concentrations (table 1) were toxic.

The results obtained with synthetic *dl*-isoleucine as compared with those obtained with two samples of the unnatural isomer of isoleucine clearly indicated that the near duplication of the symptoms of frenching was due only to the action of the natural optical isomer, *l* (+)-isoleucine. A sample of the natural isomer proved as fully effective at 100 parts per million as did the racemic mixture at 200 parts per million. It follows that only the natural isomer of isoleucine was responsible for the frenching response obtained in these and previous experiments with the racemic mixture.

TABLE 2.—*Effects of various compounds on gross morphology of tobacco seedlings—Maryland Medium Broadleaf (Robinson strain)—grown for 28 days in 200-ml. Erlenmeyer flasks under aseptic conditions*

Compound	Quantity	Appearance of seedling
	Parts per million	
None		Normal.
2-Aminopyridine	400	Do.
2-Aminothiazole	100	Light-tan necrotic spots and blotches on leaves.
5-Aminouracil	5	Apical chlorosis of basal leaves.
Calcium formate	5	Basal chlorosis of leaves with apical green crescents.
Calcium *d*-lactate	10,000	Leaves with yellow rims; white basal leaves.
Calcium 2-keto-D-gluconate	4,000	Large white blotches on leaves.
Choline hydrochloride	150	White stem and leaf midribs.
Dihydroxyacetone	1,000	Normal; small rosette.
Ethyl oxalacetate (Na)	1,000	Leaves with slight mottle and light rims.
Glucosamin hydrochloride	400	Normal.
d-Glutamine	200	Do.
Glutathione	200	Apical and marginal leaf spots.
dl-Isoleucine	200	Rosette with strap leaves; short roots.
d (−)-Isoleucine (unnatural isomer) [1]	200	Faint interveinal chlorosis in older leaves; long roots
d (−)-Isoleucine (unnatural isomer) [2]	200	Do.
l (+)-Isoleucine (natural isomer) [3]	100	Rosette with strap leaves; short roots.
dl-Malic acid	1,000	Faint interveinal leaf chlorosis.
Nicotine	175	Chlorosis of bottom leaves; retarded growth.
Potassium bisaccharate	1,000	Yellowing of leaves with age.
Sodium acetate	5	Apical chlorosis of basal leaves.
Sodium acrylate	2	Chlorosis of bottom-leaf apices and margins.
Sodium succinate	10,000	Light general mottle; large chlorotic blotches.

[1] Received through courtesy of Merck & Co., Inc.
[2] Received through courtesy of William C. Rose.
[3] Received through courtesy of William H. Stein.

DISCUSSION OF RESULTS

The production of abnormal alterations in gross morphology of tobacco seedlings is not confined to the amino acids, as is shown by the data for the salts of formic, acetic, and acrylic acids. The exact results to be expected from accumulation of a metabolic product in the tissues of the plant are, it is surmised, the resultant of the type of product that accumulates, its concentration, and the localized region of its accumulation.

The question whether near duplication of the symptoms of frenching in tobacco seedlings grown in nutrient agar containing isoleucine is due to production within the plant of the unnatural isomer has been answered in the negative. Only the natural isomer, *l* (+)-isoleucine, will so function. The unnatural isomer was practically without effect at 200 parts per million, whereas the presence of 100 parts per million of the natural isomer in the synthetic product caused strap-leaf formation characteristic of extreme frenching.

The very gradual resumption of normal growth by the tobacco seedling after treatment with a low concentration of *dl*-isoleucine indicates a normal, though limited, coupling reaction in the plant to this amino acid. Spontaneous recovery of frenched plants in the field has been observed, also.

It is doubtful whether the addition of free amino acids, etc., to a cultural medium can successfully be used to produce symptoms that are identical to those produced in a specific abnormal field environment. The reasons for this are obvious. Probably no specific syndrome results from accumulation of a single compound. It is more likely, in fact, that as yet unknown amino acids and other metabolic products participate in the causation of symptoms. The extent to which duplication of the symptoms of frenching was brought about by use of the very simple mixtures employed in the experiments described in this paper is therefore surprising. A factor that probably operated to prevent exact duplication in the experiments of any specific aggregate of symptoms is the continued normal functioning of whatever mechanisms for detoxification exist in the plant. In naturally abnormal plants certain of these detoxification mechanisms may have ceased to function. It is doubtful, finally, whether identical symptoms will be shown by seedlings and mature plants or by plants grown in flasks and plants grown in the field.

The data indicate that the effects of the free amino acids on tobacco seedlings are not confined to production of morphological abnormalities. The free amino acids tested, particularly isoleucine, also caused alterations in rate of stem and leaf growth. Stem elongation was retarded by *dl*-isoleucine at very high dilutions that permitted otherwise apparently normal development of the plant. If the property of isoleucine that tends to cause such alterations is normally utilized by the plant, the responses obtained would be subject to wide modification as a result of fluctuations in the quantities of other metabolites present. The properties of different amino acids in admixtures are not necessarily additive or the same in kind.

It appears probable that use of still longer growth periods and of quantities below the threshold values for obvious abnormal symp-

toms would result in a greater number of apparently normal morphological responses.

SUMMARY

Seedlings of Maryland Medium Broadleaf tobacco (*Nicotiana tabacum* L.)—Robinson strain—were grown in aseptic culture in 200-milliliter flasks for 28 days or in 1-liter flasks for 2 months with a complete mineral agar containing one or more amino acids or other compounds that are known or may be assumed to occur in tobacco plants. Continuous illumination of about 500 and 300 foot-candles from white fluorescent lamps was maintained. Response of the seedlings to the presence of amino acids in the medium varied with kind, concentration, and admixture over a wide range in gross morphology. In particular, response to *dl*-isoleucine in a concentration of 200 parts per million closely approximated symptoms of the disease known as frenching, including formation of strap leaves, reticular chlorosis, inhibition of stem elongation, and large increase in leaf number.

Seedlings grown in nutrient agar containing 4 or 5 parts per million of *dl*-isoleucine were retarded in stem growth but otherwise appeared to have developed normally. The unnatural isomer of isoleucine at 200 parts per million did not cause the frenching response to appear, whereas the natural isomer at 100 parts per million did. Toxicity of asparagine was six times that of glutamine. The longer growth period led to better defined toxicity symptoms and to the appearance of some new responses, a few of which fell within the range of normalcy, Acids of low molecular weight caused more pronounced symptoms of abnormality than those of high molecular weight.

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